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EDITORIAL

WHEN the first two parts of Volume XIX of this JOURNAL were published as a double number in August 1941, the Editors referred to the inevitable modification in its issue under the exigencies of war. However, they expressed their determination to continue their efforts to keep the JOURNAL in being, despite the considerable difficulties, and Volume XX duly appeared, as two double numbers, in 1942 and 1943. In preparing for issue the four parts combined as Volume XXI at the close of 1944 they feel that some explanation is due both to subscribers and contributors of papers.

It is hoped that subscribers will realize to what a great extent the thoughts and activities of horticultural scientists have been directed towards *ad hoc* problems of increasing food production, and that while as much fundamental research as possible has also been carried on, the workers have had little time to assemble their results and prepare them for publication. It is encouraging that papers have still come forward intermittently from various parts of the world, but the very fact that important contributions have continued to come from overseas has inevitably delayed the production of the present Volume. It is hoped that circumstances in 1945 will allow a return to more regular quarterly or half-yearly issues, as in pre-war days, and that contributors, while appreciating the present difficulties of rapid publication, will be able to look forward to an earlier appearance of their results as the world situation improves. The present volume is intended to bring the JOURNAL up-to-date as regards papers submitted since the publication of Volume XX.

With the retirement of Professor B. T. P. Barker from the Directorship of the Horticultural Research Station, Long Ashton, and the appointment of Dr. T. Wallace as his successor, the latter automatically assumes Joint Editorship of the JOURNAL. However, after Professor Barker's long association with it, all its readers and contributors will be glad to know that he has consented to become an Associate Editor and so will continue to put his wide experience at the disposal of those responsible for its production.

December 31st, 1944.

PROTEIN PRECIPITATION AND VIRUS INACTIVATION BY EXTRACTS OF STRAWBERRY PLANTS

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ALTHOUGH virus diseases are an important cause of degeneration wherever strawberries are grown, little is known about the number of viruses concerned or about their relationship to one another. None of the viruses or virus strains has been transmitted mechanically, so that their properties *in vitro* remain undetermined, and diagnosis rests solely on symptomatology. This is even more unsatisfactory for strawberry viruses than for those attacking most plants. Different strawberry varieties react in different ways to the same virus or virus-complex, whereas one variety may react similarly to infection with different viruses or virus strains, and the symptoms shown by one and the same plant depend greatly on its environment (Harris, 1937; King and Harris, 1942).

With many other plant viruses, serological methods have proved valuable for demonstrating relationships between virus strains that cause different symptoms and for distinguishing between unrelated viruses that cause similar symptoms. Such methods also provide a rapid means of diagnosing plants suspected to be infected, and they have been used to determine the properties of the sugar beet Yellows virus, one which is not transmitted mechanically (Kleczkowski and Watson, 1944). It seemed possible, therefore, that serological methods might usefully be applied to the study of strawberry virus diseases, but all our attempts to produce antisera against extracts of infected strawberry plants have failed. A possible explanation of this can be found in the properties of the extracts, for they differ strikingly from those of extracts from solanaceous and other plants which we have studied previously.

MATERIAL AND METHODS.

All the strawberry plants used in the work here described were kindly supplied by Mr. R. V. Harris of the East Malling Research Station. Fully developed leaves from Royal Sovereign plants, some virus free and others suffering from Crinkle, were first used. Passing the leaves through a meat mincer and squeezing the mince by hand through muslin—a method that gives an abundant supply of sap from all other plants we have handled—failed to give any juice. Even squeezing in a hydraulic press produced only insignificant quantities, for the water content of freshly picked strawberry leaves is only about 60 per cent., i.e. rather less than that of the residual " fibre " of tobacco leaves after the sap has been expressed.

To get extracts for testing as antigens, therefore, a different method was adopted. After mincing, the " fibre " was ground by passing it through a fast revolving roller mill; 10 ml. of water were then added for every 20 grm. of the leaf, the whole was then thoroughly mixed and centrifuged for 10 minutes at 10,000 r.p.m. About half the added volume of water was then recovered above the sedimented material as a viscous, brownish-yellow, transparent fluid, with a pH value of 6·2. These extracts were used to inject rabbits, six intravenous injections of 1·5 ml. each being given at weekly intervals. Eight days after the last injection, the rabbits were bled and serum was prepared.

EXPERIMENTAL.

Constitution of extracts.—No differences were found between extracts from leaves of healthy and Crinkle-affected Royal Sovereign plants. When mixed with any antisera or with normal rabbit serum, both kinds of extract caused an immediate bulky precipitate. It was obvious

that the extracts contained material that precipitated serum proteins, and they were therefore quite unsuitable for serological work. Tests were then made to find out the nature of the material and to see if extracts of leaves could be prepared free from it.

The extracts did not change in appearance when boiled and there was no precipitate when they were boiled with trichloracetic acid, so that it seemed unlikely that they contained any appreciable amount of protein. This was confirmed by micro-Kjeldahl tests, which showed less than 0·1 mg. of nitrogen per ml. The extracts had a solid content of about 8 per cent. They reduced Fehling's solution to an amount corresponding to a glucose content of 2·7 per cent. After hydrolysis by boiling with 2 per cent. hydrochloric acid for 30 minutes, with a reflux, the reduction corresponded to a glucose content of 4·3 per cent. Assuming that the total reduction is due to carbohydrate, more than half the dry weight would seem to be of this nature.

The boiled extracts smelt like tea and they gave a strongly positive test for tannin. A few drops of 5 per cent. ferric chloride solution added to 15 ml. of the extract after diluting 1 to 25 with water still gave an intense dark blue colour. Tannin could also be extracted from the minced leaves by 70 per cent. acetone. From analogy with other viruses, it is likely that strawberry viruses will prove to be proteins, or at least to contain protein, so that even if the extracts could be freed from all tannin and any other constituents that precipitate serum proteins it was unlikely that they would contain virus or other antigenic substances. Attempts were therefore made to see whether the tannin could first be removed from the leaves and then protein-containing extracts prepared. Fifty ml. of 70 per cent. acetone were added to 15 grm. of milled leaf, squeezed out and found to have a high tannin content. The acetone remaining in the fibre was evaporated, and an aqueous extract was made, but this contained no protein. Similarly, successive aqueous extracts were made of minced leaves. Only the first extracts gave a strong tannin reaction with ferric chloride and precipitated serum proteins, but the later extracts did not contain appreciable amounts of protein or of nitrogen. Thus, it seems that when strawberry leaves are minced, large quantities of tannin are released, and these precipitate the proteins, so that aqueous extracts of the "fibre" are protein-free.

Virus inactivation by strawberry-leaf extracts.—The absence of protein in extracts of strawberry leaves could in itself explain the failure of mechanical methods of transmitting viruses affecting this plant, for it is to be expected that the viruses as well as the normal plant proteins would be precipitated with the "fibre" and not extracted in any fluid expressed from the leaves. Even if a certain amount of virus was extracted, however, the properties of the extracts are such that mechanical transmission would be unlikely. When rubbed over the surface of leaves of tobacco and *Nicotiana glutinosa* the extracts cause considerable scorching and necrosis, and they effectively prevent infection with such an easily transmissible virus as that causing tobacco Mosaic.

This effect is shown in Table I, in which it will be seen that the addition of an equal volume of an aqueous extract of leaves of Royal Sovereign completely inactivated or inhibited a 0·005 per cent. solution of tobacco Mosaic virus and greatly reduced the infectivity of a 0·05 per cent.

TABLE I.

Effect of extracts of Royal Sovereign strawberry leaves on the infectivity of tobacco Mosaic virus.

Diluent.	Infectivity. Average number of lesions per leaf with virus at: 0·05%	0·005%
Water	202	78
Water extract of leaf	7	0
Acetone extract of leaf*	0	0

* Acetone evaporated and residue dissolved in water, using 1 ml. for 2 grm. of original leaf.

solution. That this loss of infectivity is probably produced by tannin in the extract is shown by the still greater inactivation produced by the redissolved material extracted from the leaf by acetone. Further evidence for this was obtained by comparing the inactivating effect of an aqueous extract with that of a solution of tannic acid. 0·002 per cent. solutions of tobacco Mosaic virus were mixed with equal volumes of variously diluted extracts of strawberry leaf and with various concentrations of tannic acid and inoculated into *Nicotiana glutinosa*. Table II

TABLE II.

Relative effects of aqueous extracts of strawberry leaves and tannic acid on tobacco Mosaic virus and Nicotiana glutinosa.

Dilution of extract.	Infectivity. No. of lesions per leaf.	Appearance of leaves.	Tannic acid concentration.	Infectivity. No. of lesions per leaf.	Appearance of leaves.
1/1	3	Damaged	1%	1·5	Damaged
1/2	2	,,	0·5%	1·5	,,
1/4	11	,,	0·25%	10	,,
1/8	23	Undamaged	0·12%	15	Undamaged
1/16	33	,,	0·06%	24	,,
Water control	48	,,	0·03%	41	,,

shows that the extract behaved, both by reducing infectivity and by its effects on the leaves, in much the same manner as a 1 per cent. solution of tannic acid.

As the concentrated extracts caused considerable damage to the inoculated leaves, the reduction in infectivity might arise mainly from an effect on the susceptibility of the host rather than from direct action on the virus. The following experiment, however, suggests that this is not so. Tobacco Mosaic virus at 0·002 per cent. was diluted with an equal volume of either water or strawberry leaf extract. Leaves of *Nicotiana glutinosa* were rubbed with virus solution, water, and leaf extract or with mixtures of virus solution and extract. They were then washed with water and dried. Leaves previously rubbed with water or with the extract were then rubbed with virus solution, and those previously rubbed with virus solution were rubbed with water or with extract. Table III shows that the extract exerted its full effect only when mixed

TABLE III.

Effect of inoculation with strawberry leaf extract and tobacco Mosaic virus at different times.

Inoculum.		Infectivity.
First inoculation.	Second inoculation.	Average number of lesions per leaf.
Extract+Virus	0	1
Extract	Virus	13
Virus	Extract	74
Virus	0	145
Water	Virus	77
Virus	Water	73

with the virus before inoculation, although it also had some effect when rubbed over leaves before inoculation with the virus. Applying the extract after inoculating with the virus had no greater effect than subsequent rubbing with water. Thus it seems more likely that the main effect is directly on the virus, rather than on the host.

The inactivating effect is not restricted to tobacco Mosaic virus and *Nicotiana glutinosa.* A tobacco Necrosis virus diluted in water gave an average of 116 lesions per leaf when inoculated into beans, whereas when diluted equally with an aqueous extract of strawberry leaves it gave only 3 lesions.

Extracts of other tissues and of other rosaceous plants.—Since the present work began, Mushin (1943) has reported unsuccessful attempts to produce antisera against strawberry Crinkle virus. She used extracts of young leaves, as she found that these did not precipitate serum proteins as did extracts of old leaves. We have therefore compared extracts of young Royal Sovereign leaves with extracts of fully developed ones, but have found no significant differences except in pH. No sap could be expressed from the minced young leaves, and extracts of them were made as described above. These were transparent, yellow and viscous, with a pH value about 4·7. They contained no detectable protein, gave a strong colour reaction for tannin, precipitated serum proteins, inactivated tobacco Mosaic virus and damaged the leaves of *Nicotiana glutinosa* in exactly the same way as did extracts from older leaves. Leaves from the wild strawberry, *Fragaria vesca,* behaved in exactly the same manner as those from Royal Sovereign. Leaves of the variety Huxley had a slightly higher water content, so that a little sap could be expressed from the mince ; otherwise they behaved like leaves of Royal Sovereign.

The water content of strawberry stolons and roots was enough to give ample sap by squeezing after mincing. After centrifuging, the juice from stolons was clear and brown, with a pH about 4·7, while that from the roots was yellow, with a pH about 6. Otherwise the properties of the two extracts were very similar. Neither contained any detectable protein and both precipitated serum proteins. Their tannin content, however, was much lower than that of leaf extracts, and they did not greatly reduce the infectivity of tobacco Mosaic virus or cause obvious damage when rubbed over leaves of *Nicotiana glutinosa.*

Tests were also made with juice extracted from ripe fruits of healthy Royal Sovereign and of Huxley infected with Yellow Edge and Crinkle viruses. The centrifuged juice from both was clear, pink and viscous, with a pH value around 4. It was highly buffered, 6 drops of Normal sodium hydroxide being needed to neutralize 1 ml., and it became brown when neutralized. The juice gave no colour test for tannin and it contained no protein, although it had a nitrogen content of 0·2 mg. per ml. The neutralized juice remained clear when mixed with serum, but the untreated juice became opalescent. In common with other fruit juices that have been tested (Johnson, 1941), strawberry juice reduced the infectivity of tobacco Mosaic virus. Table IV shows the effect on infectivity of mixing equal volumes of a 0·002 per cent. Mosaic virus

Table IV.

Effect of strawberry fruit juice on tobacco Mosaic virus.

Material added to Mosaic virus.	Infectivity. Average number of lesions per leaf.
Water	114
Royal Sovereign fruit juice	40
Neutralized Royal Sovereign fruit juice	64
Huxley fruit juice	40
Neutralized Huxley fruit juice	81

solution with strawberry juice before and after neutralization. Extracts of leaves, stolons, roots and fruits from infected Huxley plants were all inoculated into healthy Royal Sovereign and tobacco plants ; some produced local damage but none produced any infection.

DISCUSSION.

The methods by which a particular plant virus has been experimentally transmitted generally occupy a prominent place in its characterization, for they are usually regarded as indications of intrinsic properties of the virus, independent of its host. Clearly, where two viruses are known to affect the same host and one can be transmitted by mechanical inoculation and the other cannot, there is good reason to believe that the difference lies in the viruses themselves. However, the results presented here suggest that failure to transmit strawberry viruses mechanically is just as likely to be due to properties of the host as to those of the viruses. It is almost certain that all attempts to transmit strawberry viruses by inoculation have been made with fluids that contained no virus in an infective state. During maceration of the leaves it seems that the normal protein, and presumably the viruses as well, are brought into contact with tannin and perhaps other protein precipitants. These are present in such quantities that, after precipitating all the strawberry leaf proteins, aqueous extracts of the " fibre " contain sufficient to precipitate added proteins ; and they are also efficient inactivators of mechanically transmitted viruses. It is, of course, possible that the strawberry viruses could not be transmitted by inoculation even if they could be extracted from infected plants and freed from inactivating substances. This question will probably remain undetermined until the viruses have been transferred to some host that is also susceptible to viruses known to be mechanically transmissible. Until this has been done, failure of mechanical transmission can reasonably be regarded as a result of peculiarities of the host plant.

In this connection it is worth noting that more than twenty viruses attacking rosaceous plants have been described, and none of these has been transmitted mechanically. We have not examined a sufficient number of members of the Rosaceae to know if our results with strawberry have a general application, but some tests with raspberry leaves have shown that the strawberry is not unique. These leaves had a higher water content than strawberry leaves, so that sap could be expressed from them when minced. Otherwise, however, they were similar to strawberry leaves. Their sap gave negative tests for proteins ; it precipitated serum proteins and contained tannin, and it inactivated tobacco Mosaic virus, though to a less extent than extracts of strawberry leaves.

It seems most likely that the difficulties of working with strawberry viruses *in vitro* arise from the high tannin content of the leaves, but difficulties in interpreting the results of transmission tests might arise from other less easily detectable constituents. It is well known that sap from some plants, particularly that from *Phytolacca decandra*, contain substances that are very efficient in neutralizing the infectivity of mechanically transmissible viruses. Similarly, we have found that sap from hop leaves, although it contains no tannin or other protein-precipitants, and itself contains proteins, is much more effective in reducing the infectivity of tobacco Mosaic virus than is sap from raspberry leaves.

SUMMARY.

Specific antisera could not be produced against extracts of virus-infected strawberry plants, possibly because of properties of the host plant. No soluble protein could be extracted from fruit, leaves, runners and roots ; and aqueous extracts from all organs reduced the infectivity of tobacco Mosaic virus. Except those from the fruit, all extracts contained much tannin and they precipitated serum proteins. The possible effect of this on failure to transmit strawberry viruses by mechanical inoculation is discussed.

ACKNOWLEDGMENT.

Whilst carrying out the work described, one of us (A.K.) has been in receipt of a grant from the Agricultural Research Council.

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THE RELATIONSHIP BETWEEN RESPIRATION AND PHYSICAL CONDITION OF FRUIT AS AFFECTED BY OIL TREATMENTS

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INTRODUCTION.

A FACTOR of great importance in determining the quality of fruit is the expressible juice content. Thus, Reyneke (1941) has shown that woolliness in Peregrine peaches depends on the quantity of juice liberated when a constant external pressure is applied to a standard quantity of tissue. The amount of juice obtained is an expression of the physical condition of the tissues and is largely determined by the number of cells ruptured. Many undesirable conditions such as woolliness in peaches, mealiness in apples, pears, potatoes and tomatoes are associated with a low juice content. In view of the importance of a high juice content to the consumer and the manufacturer of by-products, a study is being made of the factors affecting it, and in the present investigation particular attention has been paid to the relationship between the rate of respiration and the juice content of the fruit.

EXPERIMENTS WITH BON CHRÉTIEN PEARS.

Picking A.—Fruit was obtained from healthy, mature trees. The pears were picked at the usual commercial picking period, immediately acid washed and graded. A medium size ($2\frac{1}{4}$-$2\frac{3}{4}$ in.) was selected for experimental purposes, and all blemished fruits were rejected.

Picking B.—This consisted of pears gathered from old, stunted trees bearing small leaves, and growing in a locality exposed to the wind. The fruit was picked and handled as above, and the same grade was used.

METHODS.

Preparation of the fruit.—After the fruit had received its various treatments, which are detailed under the individual experiments, it was wrapped and boxed in bushel boxes. These were stored in a room controlled to give a temperature of 58° F. Respiration determinations were carried out with 4-6 fruits depending on their size. The same fruit was then cut and sliced to give slices 1·5 mm. in thickness for physical and chemical determinations.

Respiratory activity.—A conductivity method was used. Air freed from carbon dioxide was passed through a desiccator sufficiently large to contain the fruit with little dead space, and then through a conductivity cell similar to that described by Newton (1935). The method of operation is fully described in the paper referred to and need not be repeated here.

Expressible or free juice content.—100 grm. of the sliced tissue were carefully placed in the centre of a perforated tincture press container closed at one end. A lid made to fit the container was then pushed down on to the sample. Increasing pressure was then slowly applied at short intervals, until one was reached after which further increase yielded no more juice. As the fruit matured, increased amounts of tissue were forced through the perforations in the container. The expressed juice, or the pulp, collected in the outside container was wiped on to a piece of cheese cloth and carefully squeezed between the fingers to separate the juice from the pulp. The juice was then centrifuged to remove suspended particles, and the volume measured; differences, not exceeding 0·5 c.cm., between duplicate samples were obtained.

Acidity.—10 c.cm. of the expressed juice, diluted to approximately 300 c.cm., was titrated with N/10 NaOH, using phenolphthalein as indicator.

Acetaldehyde.—150 grm. of the sliced tissue, frozen at $-15°$ C. for 48 hours, were placed in a 750 c.cm. distillation flask and steam distilled until a distillate of approximately 500 c.cm. had been collected. The distillate was passed through ice cooled condensers, and collected in ice cooled receivers containing 50 c.cm. of a N/10 sodium bisulphite solution. The excess sulphite was titrated with N/10 iodine, using a starch solution as indicator.

Preparation of oil emulsions.—The emulsions used in the experiments with pears were prepared by mixing 570 c.cm. of oil with 10 c.cm. of oleic acid, and this mixture was slowly added to 150 c.cm. of 10 per cent. KOH solution containing 15 grm. of casein. The resulting emulsion was used either for the direct wiping of pears or for further dilution to the required oil concentrations. For apples, the emulsions were prepared by adding 2-3 c.cm. of oleic acid to 100 c.cm. of oil and neutralizing with 30 per cent. KOH solution, using phenolphthalein as indicator. The required concentrations were obtained by dilution.

RESULTS.

A. EXPERIMENTS WITH PEARS OF THE A PICKING.

1. *Relationship between juice content and rate of respiration.*—In Fig. 1, changes in the rate of respiration and juice content for untreated pears and pears of the A picking wiped with the concentrated oil emulsion are shown. From this it is clear that a high general level of respiration is associated with a low juice content. Thus, the untreated pears, showing a high respiratory activity and a high peak rate at the climacteric, never attain a high juice content, while the oil treated fruits, with a much lower respiratory activity, attain a much higher juice content.

A more detailed study of the relationship between juice content, respiration rate, firmness and starch content of Bon Chrétien pears kept at 72° F. was made, sampling being more regular and at shorter intervals. Changes in the rate of respiration, juice content and firmness are shown in Fig. 1A.

According to the changes in juice content, three phases are recognized. During the first phase the juice content falls to a minimum, while the respiration rate rises rapidly ; and these changes are associated with a rapid softening of the tissue, as measured by a $\frac{5}{16}$ in. U.S. pressure tester. The second phase is characterized by a rapid rise in the juice content to a maximum, while the rate of respiration attains its climacteric peak and the post climacteric fall begins. The firmness of the fruit decreases much more gradually during this phase, the starch—as indicated by treating the exposed surface of a fruit, cut transversely, with a 1 per cent. iodine solution—disappears when the climacteric peak is attained ; and the fruit reaches its prime eating condition shortly afterwards, i.e. with the onset of the post climacteric and maximum juice content. The third phase is the senescent one, during which respiration rate, juice content and firmness decrease together. At the beginning of this phase senescent scald makes its appearance.

It is clear that factors affecting the respiration rate of Bon Chrétien pears are closely related to changes in the physical condition of the fruit, as indicated by its expressible juice content and firmness. The precise nature of this relationship will be dealt with more fully in a future communication.

2. *Effect of different oils on the rate of respiration and juice content of Bon Chrétien pears.*—Samples of pears, each consisting of two bushels, were carefully wiped with the concentrated standard emulsion of the following oils and fats : mineral, maize, Soya bean, olive, Arachis and butter, in such a manner as to ensure a good covering of oil, uniformly distributed. The changes in the rate of respiration and juice content of these samples and of untreated controls during storage at 58° F. are presented in Fig. 2.

It is evident from the curves that the effects of the different oils on the rate of respiration and juice content, during the three climacteric phases, were very similar to the effects just

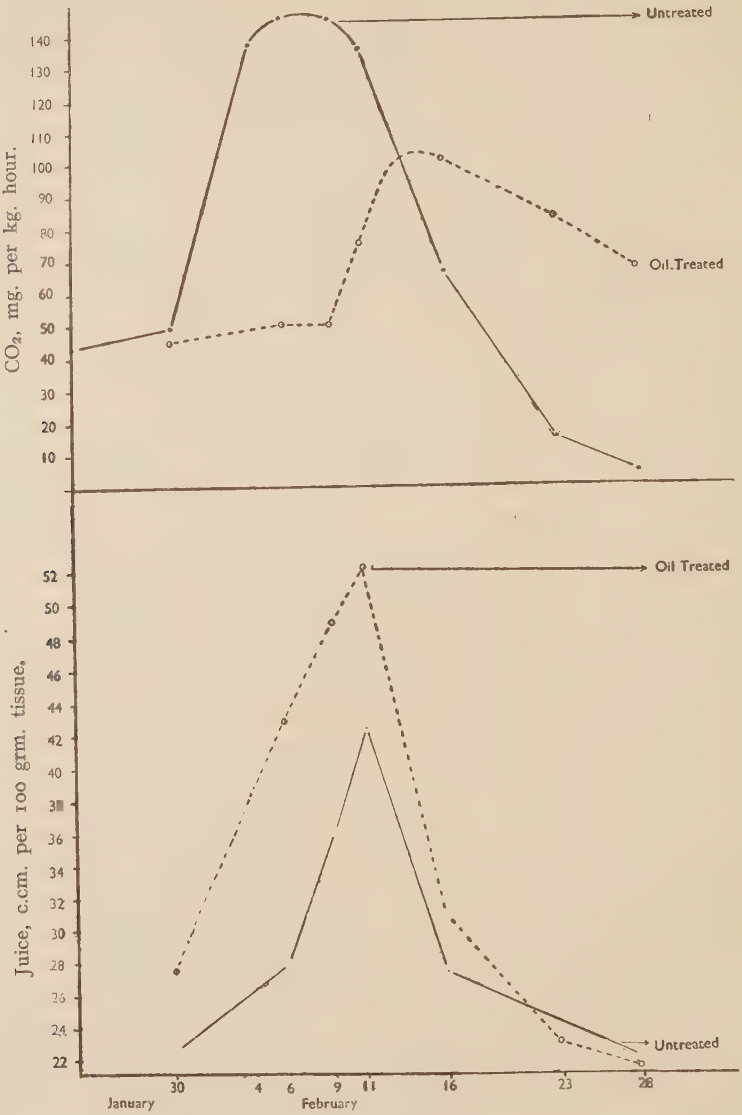

Fig. 1.

The relationship between juice content and rate of respiration of untreated and oil treated pears stored at 58° F.

described. The delayed climacteric rise—as compared with the control—was accompanied by rapid and significant increases in the juice content. The lowered respiratory peaks and the gradual decline in rate of respiration during the post climacteric period, were followed by similarly higher maximums in juice content, which were maintained over greater lengths of

time. It is furthermore clearly indicated that the general level of juice content is inversely associated with the general level of respiration.

The order in which the fats and oils depressed the rate of respiration was as follows : Arachis,

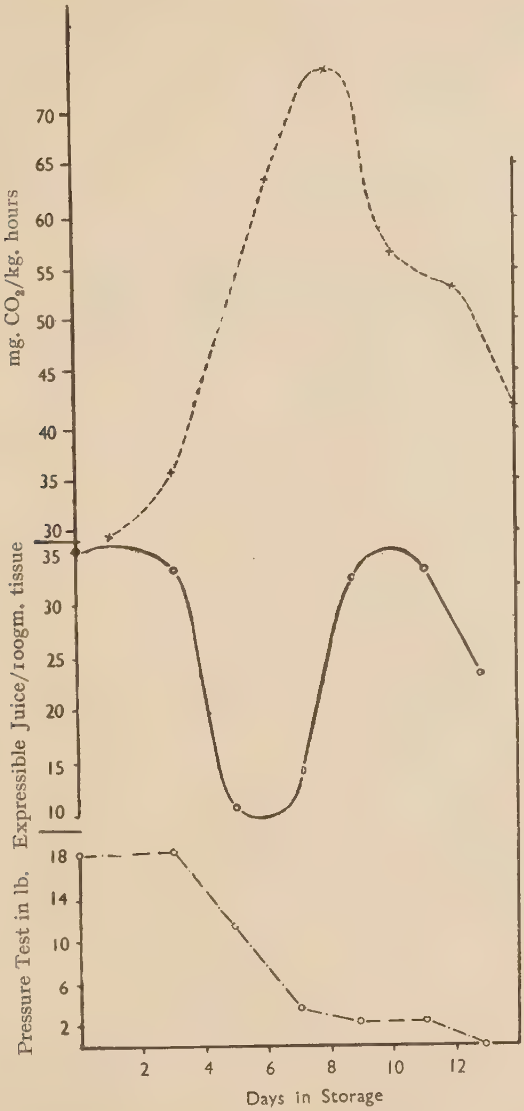

FIG. 1A.

Changes in the rate of respiration, juice content, and firmness of Bon Chrétien pears stored at 72° F.

olive, mineral, Soya bean, maize. The juice content, on the other hand, was increased and maintained at the higher levels—with one exception—in practically the same order, viz. Arachis, olive, butter, Soya bean, mineral, maize. It is interesting to note that the effect of the various vegetable oils on respiration varied in accordance with their degree of saturation as indicated

by the iodine value. The non-drying oils, Arachis and olive, iodine value varying between 83 and 105, are more effective than the medium drying oils, Soya bean and maize, iodine values ranging from 113 to 125. Unsaturated oils probably lose their effectiveness as the absorption

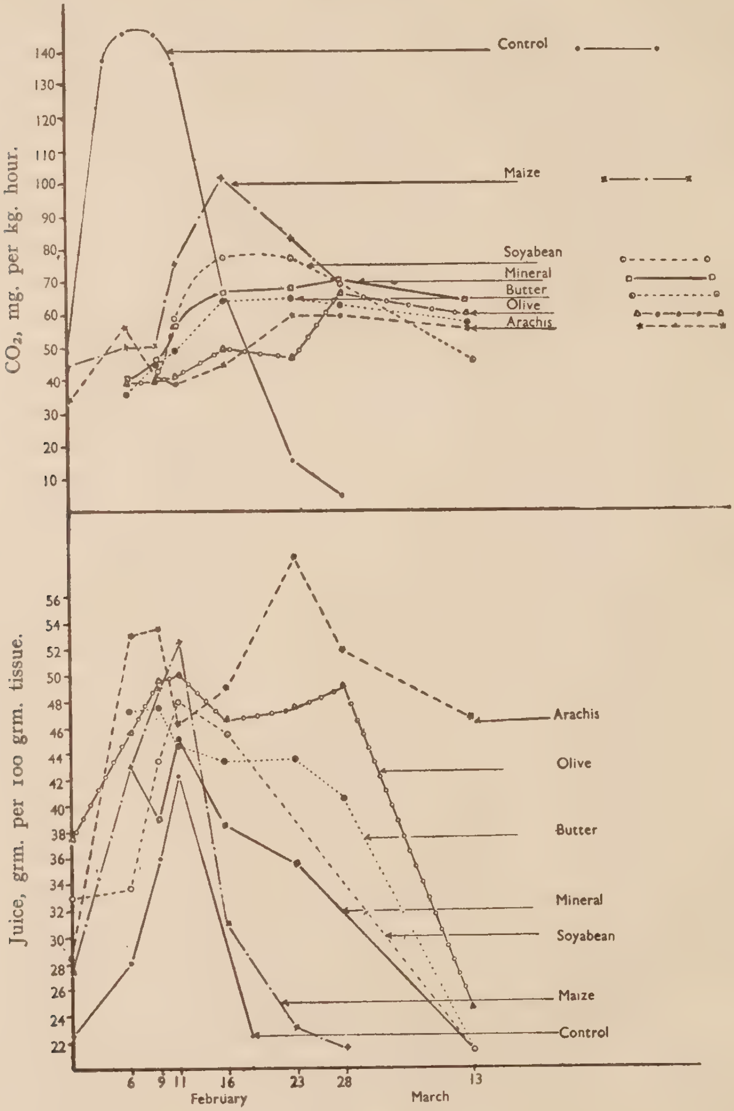

FIG. 2.

Changes in respiration and juice content of oil and fat treated Bon Chrétien pears stored at 58° F.

of oxygen and subsequent formation of resinous compounds proceeds. This property might be important in cases where rapid post-storage ripening is desired. Maize oil treated pears developed an attractive yellow colour with prominent red blush, whereas Arachis oil treated pears were of superior eating quality on account of their juiciness.

3. *Effect of growth hormones on the rate of respiration and physical condition of pears from the A picking.*—Traub (1938) reported experiments with fruits of *Passiflora edulis*, *P. antiguensis*, tree tomato (*Cyphomandra betacea*), grapefruit (*Citrus grandis*), sweet orange (*C. sinensis*),

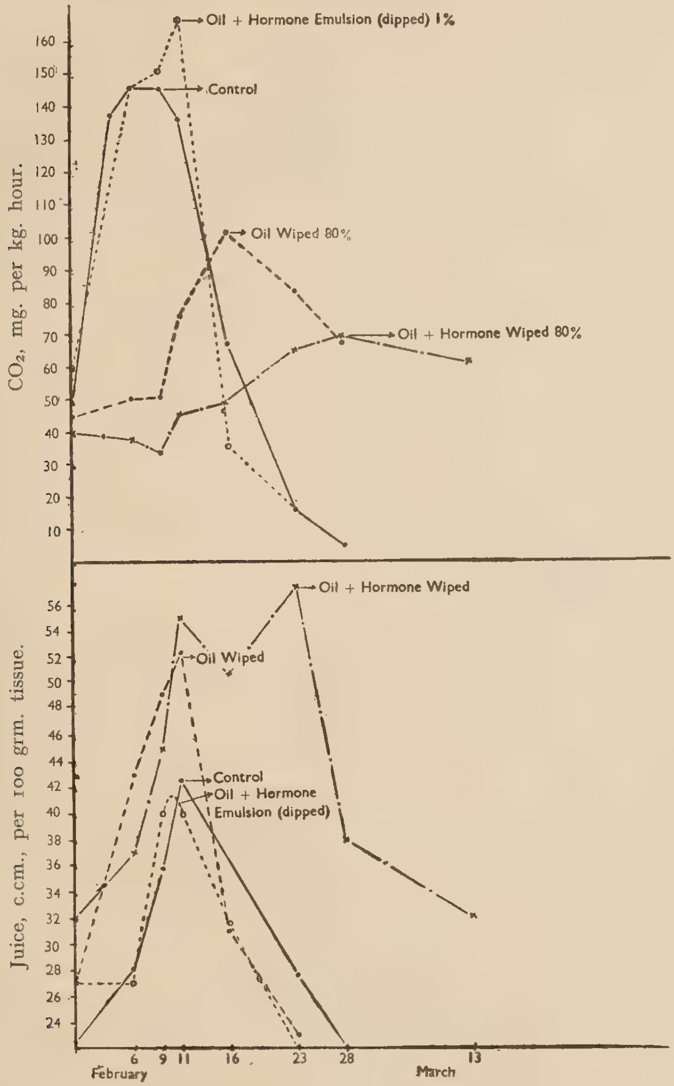

FIG. 3.

Effect of hormone on rate of respiration and juice content of Bon Chrétien pears stored at 58° F.

lemon (*C. limonum*) and lime (*C. aurantifolia*), and claimed that appropriate treatments with watery solutions or lanolin pastes of indolylacetic acid, indolylbutyric acid and alpha-naphthalene acetic acid prolonged the storage life of these fruits.

In the present experiments maize oil emulsions containing indolylacetic acid were used. The hormone was first added to the oil and the mixture emulsified. The resulting emulsion,

which contained 80 per cent. of oil and 0·1 per cent. hormone, was used as such for wiping individual pears. A lower concentration was obtained by diluting this emulsion with water

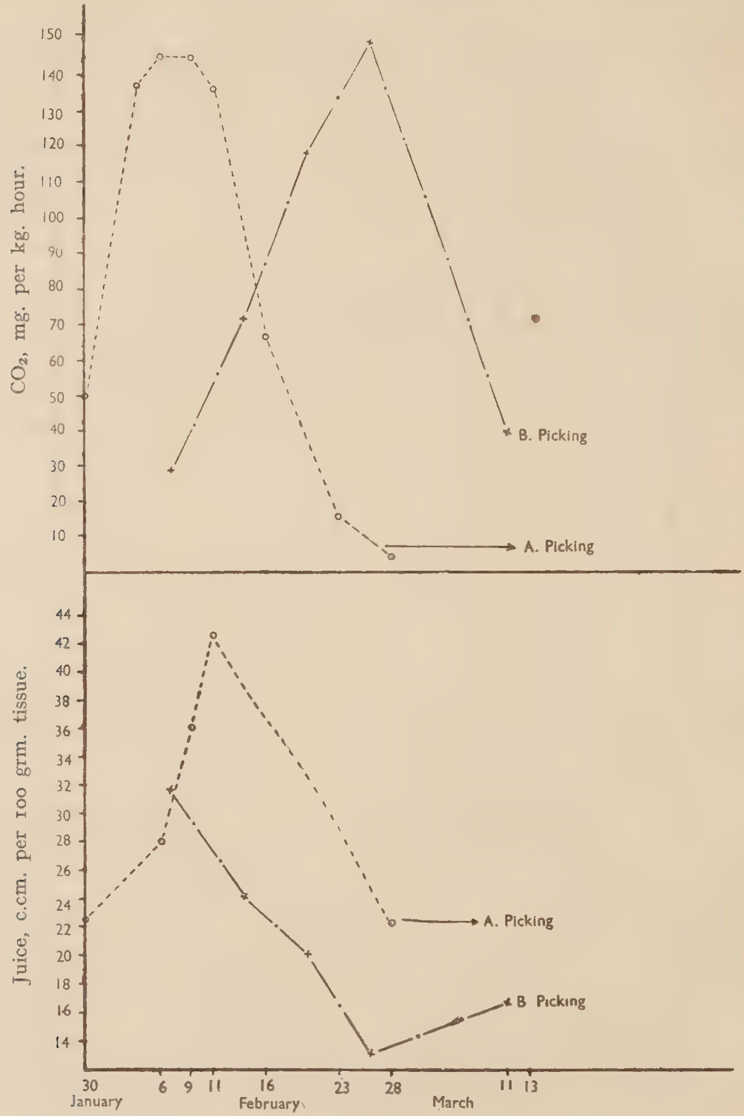

FIG. 4.

Changes in the juice content and rate of respiration of Bon Chrétien pears from A and B pickings stored at 58° F.

to 1 per cent. of oil. The effects of these two concentrations of hormone on the respiration and variations in juice content during storage at 58° F. are presented in Fig. 3.

The outstanding feature of these results is the virtually opposing effect of dilute and concentrated hormone-oil emulsions on the respiratory activities and changes in juiciness of the

fruit. The dilute emulsion had little effect, although the respiration rate appeared to be slightly increased and the juice content slightly lowered. This was in accordance with visual evidence that such pears decayed more rapidly than untreated fruit. The concentrated oil-hormone

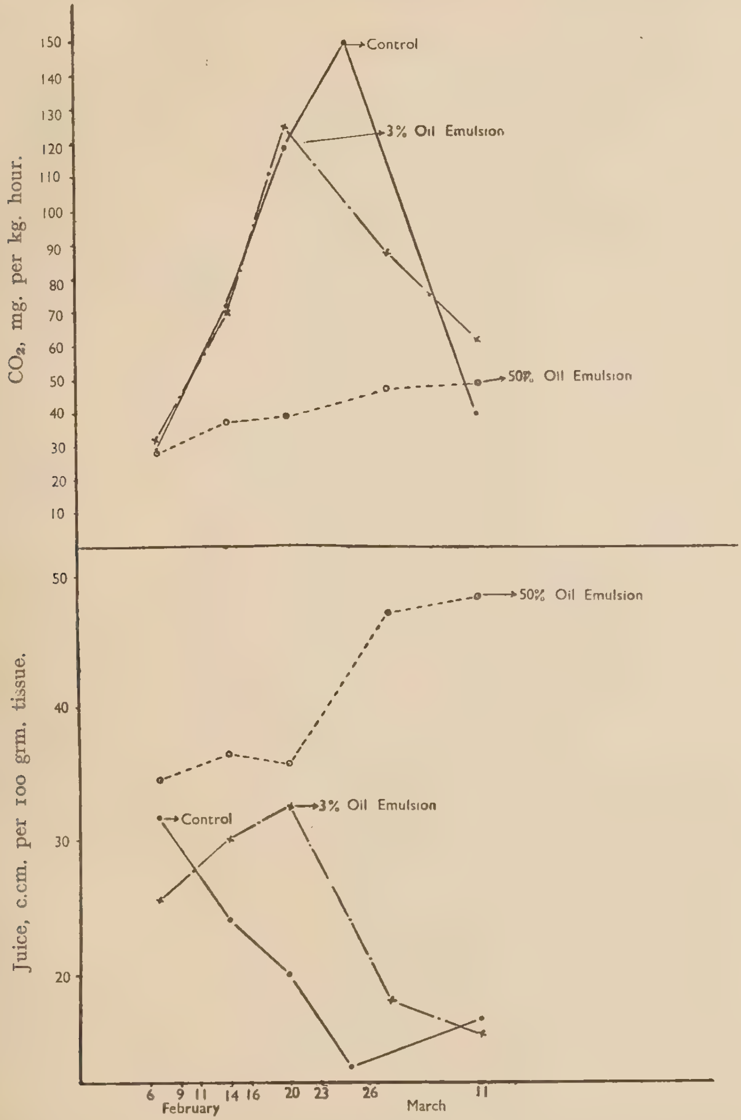

FIG. 5.

Effect of oil treatments on juice content and rate of respiration of pears from second picking stored at 58° F.

emulsion, as compared with concentrated oil emulsion without hormone, reduced the rate of respiration considerably, while the juice content increased accordingly.

It is interesting to note that the semi-drying maize oil, applied in the same manner and

strength, became, as a result of the addition of the hormone, even more effective in retarding respiration, etc., than the non-drying oils. (See Fig. 2.) Whether this effect is due to the high hormone concentration, or to an interaction between oil and hormone, or both, cannot be deduced from these preliminary results.

B. Experiments with pears of the B picking.

These pears were included in this study with the object of comparing changes in respiration and juice content of apparently normal and sub-normal fruit, and to determine whether a normal condition could be induced by various treatments designed to control respiration. A comparison of the changes in respiration and juiciness of pears from pickings A and B which occurred during storage at 58° F. are presented in Fig. 4.

From the curves it will be noted that the rate of respiration of B picking pears, as compared with that of A picking pears, increased relatively more slowly during the climacteric, but continued to do so over a longer period until finally a higher respiratory maximum was reached. Differences in changes in juice content are more striking. Contrary to the maximum in juice content reached by normal A pears, the juice content of B pears proceeded to decrease immediately after picking, and continued to do so. They never reached a condition of good eating quality, and eventually became mealy when fully coloured and ripe. Full ripeness lasted only for a short period, as severe scalding became prevalent. These pears became extremely mouldy towards the end of the storage period, probably on account of their poor and deteriorated physical condition. This is probably the type of pear, often referred to as pears of a short canning life, which becomes " floppy " in the tin.

These pears, therefore, provided valuable material for testing the effectiveness of various treatments in producing an improved physical condition which can be retained over longer periods at full ripeness. The effect of oil was determined by immersing two bushels of these pears in two different concentrations of different oils, and storing at 58° F. The changes in respiration and juice content of pears dipped in 3 per cent. and 50 per cent. maize oil only are presented in Fig. 5, the effect of other oils being essentially the same.

The results indicate that the 3 per cent. oil emulsion sufficiently retarded the rate of respiration to restore a somewhat normal curve of changes in juice content, which increased to reach a maximum at full maturity. With 50 per cent. oil emulsion the rate of respiration decreased to such an extent that the pears became extremely juicy and palatable. At full maturity this oiled sample yielded approximately 34 per cent. more expressible juice than the control. With the application of increased concentrations of oil, juiciness and palatability are promoted, whereas colour development appears to be retarded and even partially or entirely prohibited. This naturally suggests that processes concerned with the colouring of fruit are actually dissociated from those which determine physical condition, maturity or eating quality.

In this connection a preliminary experiment was carried out. Fruit was placed in a constant temperature oven kept at 86° F. almost filled with pears, and with very restricted ventilation. Changes in the rate of respiration and juice content of these pears as compared with those kept at 58° F. are presented in Fig. 6. Although the combination of factors operating was not precisely known, it is evident that the rate of respiration was significantly reduced, resulting in a high juice content increasing to a high maximum.

These pears, in contrast to those kept at 58° F., became extremely juicy, highly palatable, and developed a full yellow attractive colour. It may tentatively be suggested that the effective factors in this case were the high temperature, high humidity and high CO_2 concentrations.

The highly juicy condition was not maintained over such a long period as with heavily oiled pears, but it lasted long enough to allow time for use for various purposes. Scald development was noticed after 21 days and occurred in isolated spots, but was not so severe as with pears stored at 58° F. and brought to room temperature.

It is clear that the relationship between rate of respiration, juiciness and physical condition is important in view of the deciding effects of pre-ripening conditions on the canning quality of pears. It is known, for example, that fruit ripened under high respiratory conditions in the

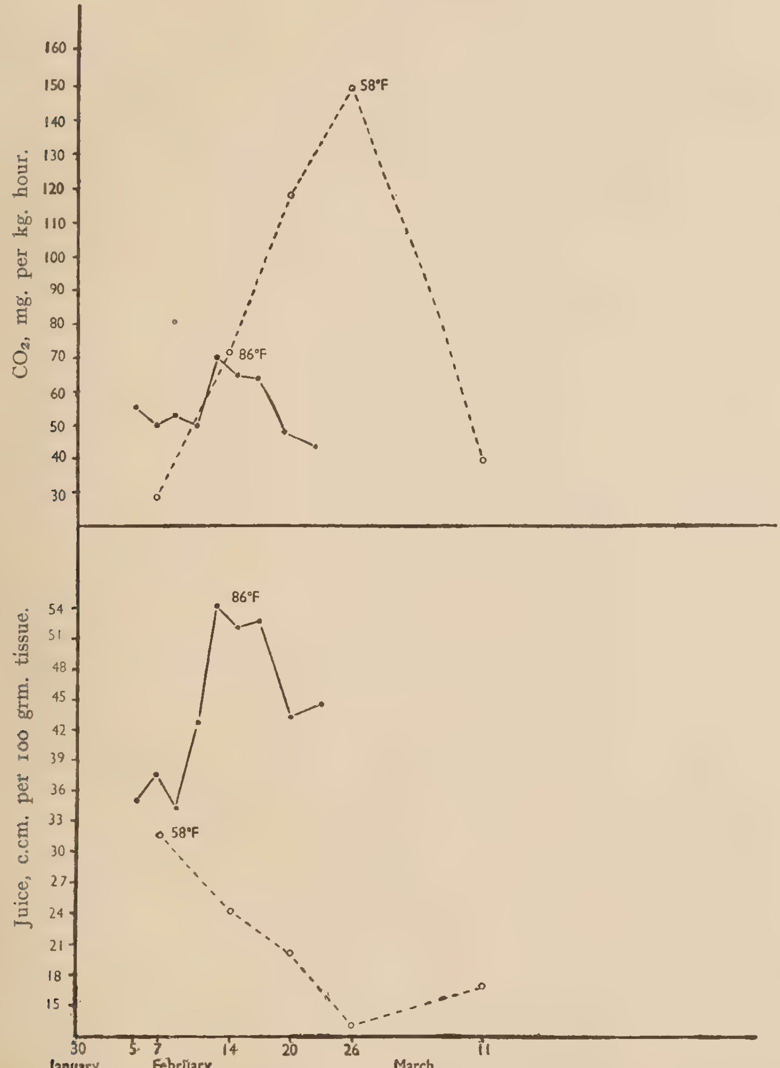

FIG. 6.

Effect of high temperature and CO_2 on the free juice and respiration rate of Bon Chrétien pears of the B picking.

orchard is not suitable for canning purposes. Furthermore, fruit ripened in a box at room temperature, as compared with that ripened on the tree, is generally more juicy and palatable. These results also indicate that juice content might be developed into a useful instrument for

determining what and when fruit should be used for various purposes. It might possibly be developed into a quantitative method of gauging effects of various treatments on the quality and suitability of fruit for various purposes.

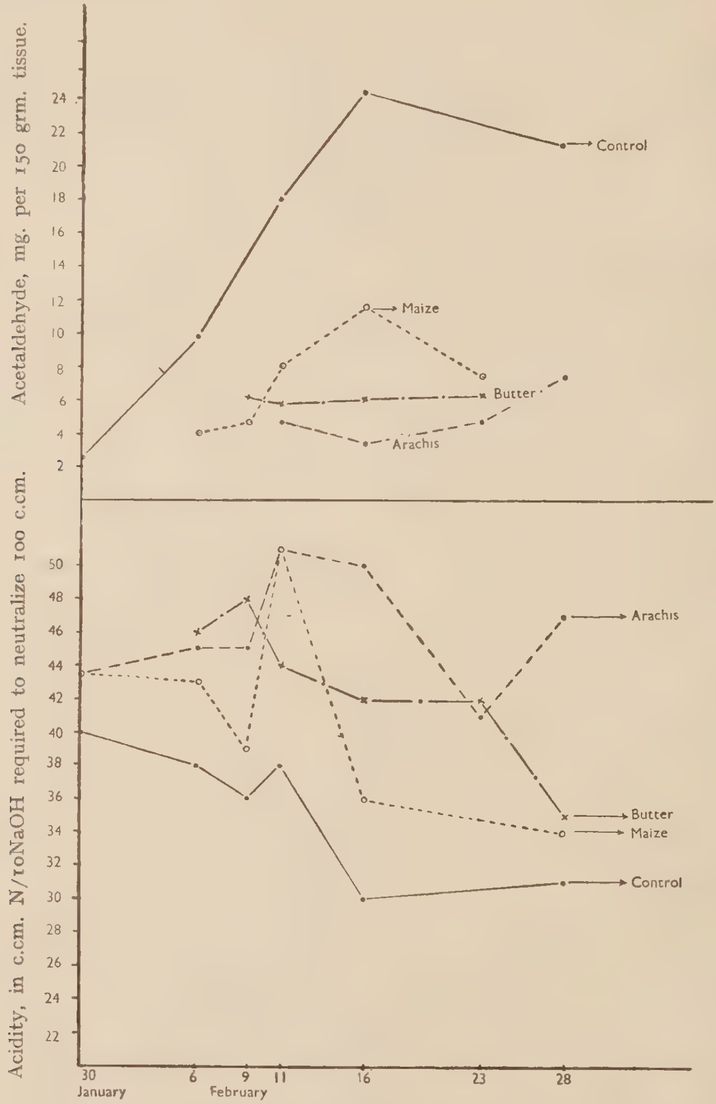

FIG. 7.

The relationship between changes in acidity and acetaldehyde content of pears stored at 58° F.

C. CHANGES IN ACID AND ACETALDEHYDE CONTENT IN RELATION TO THE DEVELOPMENT OF SCALD.

Changes in the acid and acetaldehyde content of the A pears (referred to in Fig. 2) affected by a number of the outstanding oil treatments are presented in Fig. 7.

A general inverse relationship in which low acidity is associated with high acetaldehyde content is quite evident. The order in which this relationship is affected—in terms of acidity—is: Arachis, butter, maize, control; and the acetaldehyde content consequently varied in the

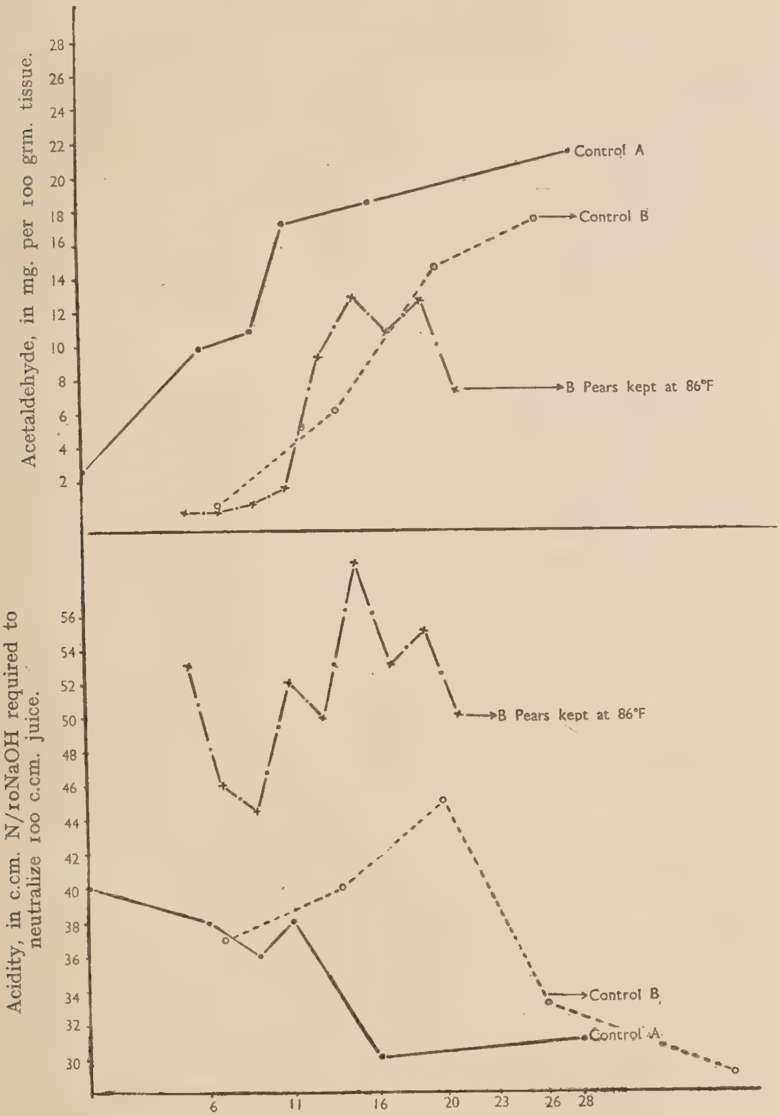

FIG. 8.

Showing relationship between changes in acidity and acetaldehyde content of untreated samples of A and B pickings kept at 58° F., and B pears kept at 86° F.

order: control, maize, butter, Arachis. These effects, compared with the respective rates of respiration and with juice contents in Fig. 2, indicate that the rate of respiration is directly associated with the rates of acetaldehyde production and acid reduction. In other words,

a high rate of respiration is associated with low acidity, high rate of aldehyde production, low juice content and a poor or rapidly deteriorating physical condition.

These observations are confirmed by comparison of the changes in acid and acetaldehyde content of pears from the B picking stored at the two different temperatures, as presented in Fig. 8, and no further discussion is needed.

In the course of storage, pears undergoing the various oil treatments referred to in Fig. 2 developed senescent scald in the order : control, maize, Soya bean, mineral, butter, olive and Arachis, the control (untreated) pears being the first and the Arachis oil treated pears the last to develop this scald. In all cases its appearance was first noticed during the post climacteric phase, and it was therefore in every case preceded by a certain amount of breakdown, as is indicated by a low or reduced juice content. These findings appear to support the view that this scald is preceded by senescent breakdown, and that its appearance is accelerated by conditions which favour a relatively high rate of respiration and a low juice content or poor physical condition. This being the case, the effect of acetaldehyde is probably of a secondary nature. Trout (1930) suggested that the most likely chain of causation (of scald) is : breakdown—water-logging of tissue—anaerobic conditions with CO_2 accumulation—aldehyde formation, rather than : aldehyde formation—toxic action—breakdown, as had been suggested by other investigators.

Further evidence in support of this view is furnished by the nature of the changes which take place during the first few hours following the removal of fruit from low to fairly high temperatures. It was noticed with these samples—in fact it is a common experience—that if fruit at a certain stage of development is transferred from low to relatively high temperatures, scald develops rapidly. Changes in the acid, acetaldehyde and juice content which occurred in 12 hours after fruit under two different treatments had been transferred from 58° F. to 86° F., are presented in Table I, in which the results indicate that significant changes occurred in the juice and acid content, whereas a similar change in acetaldehyde content was not observed.

TABLE I.

Changes in acid, acetaldehyde and juice content induced in 12 hours by transference from 58° F. to 86° F.

Treatment.	Immediately after storage at 58° F.				After 12 hrs. at 86° F.			
	Free juice content. %	Aldehyde. mg./100g.	Acid in 100 c.cm. juice. N/10 NaOH (c.cm.)	Scald.	Free juice content. %	Aldehyde. mg./100g.	Acid in 100 c.cm, juice. N/10 NaOH (c.cm.)	Scald.
Control ..	29	28·6	45	Nil	15·5	28·8	29	Severe
3% oil emulsion ..	29·5	22·2	51	Nil	22·5	20·2	34	Medium
50% oil emulsion ..	35·0	6·2	45	Nil	28·0	7·9	40	Nil

The scald which developed (especially of the untreated pears) indicates a relationship between scald and breakdown rather than one between scald and acetaldehyde content. Furthermore, a sudden drop in acidity has already been observed to coincide with rapid breakdown. (See Figs. 7 and 8.) It is possible, though, that the already high concentration of acetaldehyde in the control became fatal at the higher temperature. A secondary or contributing effect of acetaldehyde is of course under these circumstances quite possible. Whatever the secondary

effects of acetaldehyde may be, the results point in all cases towards breakdown as the necessary precursor to scald development.

The close association between acetaldehyde content and liability to scald, found by sporadic determinations, is naturally misleading. Since the rate of living, or length of life, and the rate of aldehyde production are both associated with the rate of respiration, differences in aldehyde content and in the amount of scald developed in any number of samples at a given moment, might be due to their relative stages of development. Hence, a fast living fruit, as compared with a more slowly developing one, will, at a given moment, by virtue of its relatively advanced state, contain more aldehyde and will, as a result of the physically deteriorated condition, be more liable to scald.

Scald is therefore probably the natural consequence of death and subsequent browning of cells, which under many circumstances may appear premature, as judged by physical conditions which ordinarily accompany death. The ameliorating effects of various treatments are probably entirely due to the retarded rate of respiration, improved physical condition and therefore delayed senescent breakdown. On the other hand, it is quite probable that the development of scald or destruction of cells is, under certain conditions, accentuated by the rapidly increasing concentration of aldehyde, a condition which obviously accompanies a high rate of (respiration) living.

D. Effect of orchard and pre-packing treatments on the rate of respiration, juice content and liability to scald.

In a previous article (Reyneke and Stubbings, 1940), dealing with the effects of oil sprays and of different methods employed for the removal of arsenic on the keeping quality of Bon Chrétien pears, it was shown that storage life was seriously reduced and scald development accelerated by the indiscriminate application of certain methods or reagents designed for the removal of arsenic. Treatments which injure (HCl) or partially remove the natural waxy covering, such as wetting agents (Areskap), are cases in point.

The experiments elaborated during the past season (1941) so as to include measurements of changes in respiration, juice content, etc., confirmed the previous findings. Bushel samples of pears from the B picking, treated with a solution containing 0·25 per cent. Areskap and 2 per cent. HCl, were stored at 58° F. Changes in respiration and juice content during the first fortnight, which satisfactorily reflect the general trend in changes during the whole storage period, are tabulated in Table II.

TABLE II.

Effect of Areskap and hydrochloric acid on firmness, juiciness and rate of respiration of Bon Chrétien pears stored at 58° F.

Treatment.	Firmness (pressure tests lbs.).		Juice per 100 grm. tissue (c.cm.).		Respiration per kg. hour (mg. CO_2).	
	7/2/41	14/2/41	7/2/41	14/2/41	7/2/41	14/2/41
Control (untreated)	22·8	11·2	31·6	24	28·8	71·4
Dipped in 0·5% Areskap	22·3	9·1	34·0	17	36·8	83·9
Dipped in 0·5% Areskap+0·5% HCl	22·9	5·0	27·0	9	49·4	97·1

From these results it is quite evident that as the severity of the treatment increased, the rate of respiration also increased and the drop in juice content accelerated. The Areskap and acid treatment induced early scald which spread rapidly. Breakdown or mealiness became

so severe that the fruit was barely edible at any time, and it eventually became extremely mouldy.

Similar preliminary studies were extended to include the effects of various spray materials. Pears were collected from trees which had been :

(i) Sprayed regularly with lead-arsenate according to the recognized programme.
(ii) Sprayed regularly with mineral oils instead of arsenate.
(iii) Unsprayed.

The rate of respiration after 21 days in cold storage and the percentages of superficial scald which ultimately developed are presented in Table III, and the results indicate that pears from oil sprayed trees were less susceptible to scald and respired more slowly during storage than fruit from arsenate sprayed and from unsprayed trees.

TABLE III.

Effect of sprays on the development of superficial scald, rate of respiration and aldehyde content.

Orchard treatment.	Severe superficial scald. %	Respiration after 21 days storage. mg./kg. hour CO_2.	Aldehyde content after 21 days storage. mg./100g.
Unsprayed	91	117·4	39·0
Arsenate sprays	88	107·4	39·8
Oil sprays	27·6	84·6	6·1

The retarding effect of oil sprays on the rate of ripening of fruit in the orchard and the subsequent reduced susceptibility to scald, was also indicated by Reyneke and Stubbings (1940). This effect evidently persists even after picking and during storage. Similar effects of the rate of development in the orchard on the rate of development in storage and subsequent susceptibility to various orchard and storage disorders were found by Reyneke and du Toit (1940) in Kelsey plums. Growth or orchard conditions may therefore seriously limit the effectiveness of various methods designed to increase the length of life of the fruit, and hence delay the onset of various physiological disorders.

EFFECT OF OIL TREATMENTS ON THE RATE OF RESPIRATION, PHYSICAL CONDITION AND KEEPING QUALITY OF GOLDEN DELICIOUS APPLES.

Thirty bushels of graded and packed Golden Delicious apples were taken at random from a commercial picking. The various treatments mentioned below were applied immediately the samples reached the laboratory. However, approximately seven days elapsed between picking and treatment.

Samples consisting of two bushels of fruit each, were dipped in different concentrations of various oils, while others were wrapped in wrappers containing different amounts of the various oils. Untreated controls were included. The emulsions were prepared by adding 2 c.cm. of oleic acid to each 100 c.cm. of oil, and neutralizing with 30 per cent. KOH solution. Only two concentrations, 10 per cent. and 20 per cent., were used in these tests. The oil wrappers were prepared by applying the oil to the wrapper in the form of a fine mist, by means of a spray pump. The quantity of oil per wrapper was varied by stacking the oiled wrappers alternately with untreated ones, the stack being left for three weeks to attain equilibrium.

Of the various treatments only a few were selected for detailed studies of respiration and changes in physical and chemical composition. No material difference in effect was observed between a 10 per cent. and a 20 per cent. oil emulsion, hence averages of these two treatments

and the effect of one oil wrapper on the respiration and changes in juice content are presented in Fig. 9.

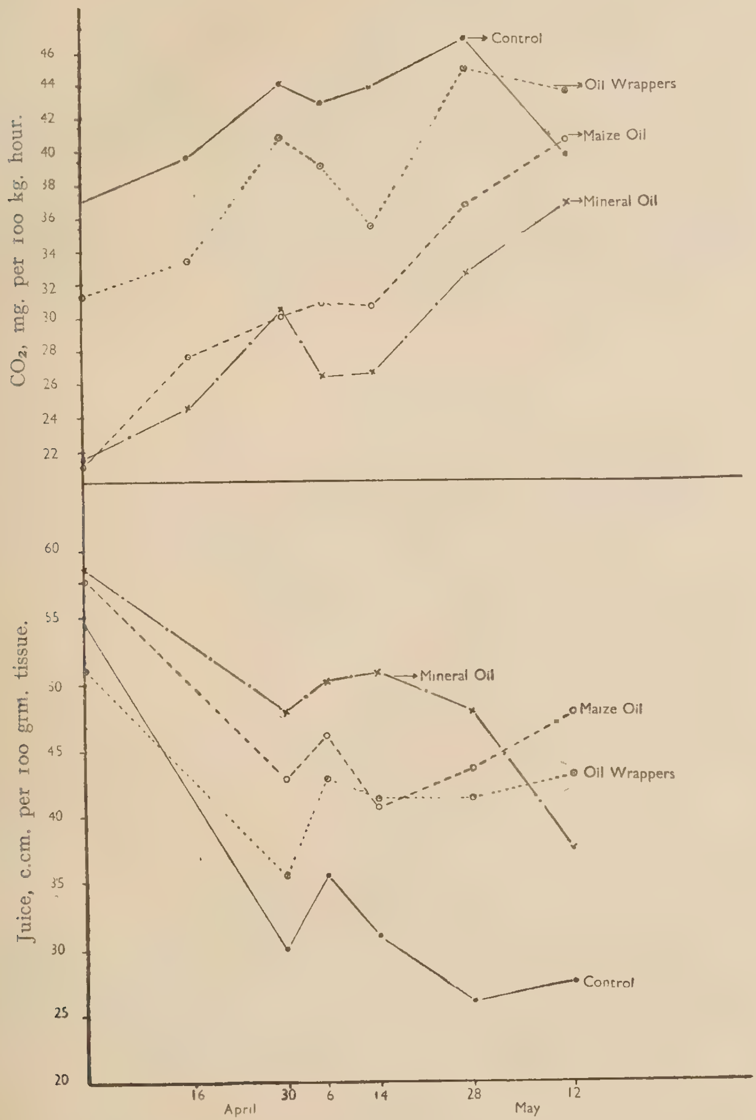

FIG. 9.

Effect of different oils on the respiration and juice content of Golden Delicious apples during storage at 58° F.

These results again clearly indicate an inverse relationship between rate of respiration and juice content. The gradual increase in rate of respiration during storage is accompanied by a similar decrease in the juice content. The different treatments compared indicate that a generally high rate of respiration is associated with a low juice content. The order in which

the rate of respiration was retarded, and the consequently higher juice level maintained, by the various treatments, was as follows : mineral oil emulsion, maize oil emulsion, maize oil wrappers, control. The order of effectiveness of the two oils was the same as that observed in pears.

It is interesting to note that no sudden rise in juice content followed the application of oil, as was observed with the pears. This is obviously due to the fact that the oil was applied during the climacteric or post-climacteric phases, and not during the pre-climacteric phase. The amount of flavour and colour which had already developed when these apples were treated, as well as the trend of changes in juice content, indicated that this fruit had already attained its post-climacteric phase when the various treatments were applied. The application of oil during this phase, therefore, exerts a preserving effect on the physical condition of the fruit only by retarding the rate of respiration.

The preserving effect exerted by the various treatments on the physical condition of the fruit was strikingly illustrated by the amount of shrivelling, breakdown and mouldiness which

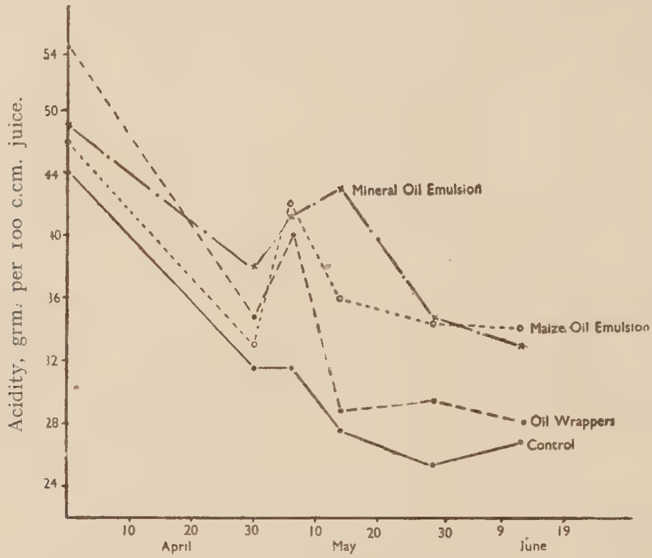

FIG. 10.

Changes in acidity of apples stored at 58° F.

developed after ten weeks of storage. The order—progressively—in which these disorders became visible on fruit from the various treatments was : control, oil wrappers, maize, mineral oil. Even after six months storage at 58° C., the amount of shrivelling, breakdown and mouldiness in fruit dipped in mineral oil was negligible, as compared with untreated fruit which had almost entirely wasted. A fact which should not be overlooked is the relative importance of the physical condition, as indicated by the juice content of the fruit, in resisting the development of mould. It is also a matter of common experience that fruit becomes liable to attack by fungi only after a certain stage of maturity is reached. This stage probably coincides with a certain state or physical condition.

These results, although of a preliminary nature, indicate that the control of most storage problems, whether due to physiological derangements or fungus diseases, largely depends on the successful maintenance or preservation of the physical condition of the fruit, which is obviously accomplished by controlling the rate of respiration. Apart from the well-known effect of oiled wrappers in controlling superficial scald in apples, the wrappers appear to exert quite significant

effects on the rate of respiration and on juice content, thus helping to delay the onset of senescent breakdown.

It will be noted from Fig. 9 that the untreated samples as compared with oil wrapped ones were, after two months storage, in a much more deteriorated physical condition—as indicated by the juice content—and obviously nearer to that point of breakdown where senescent scald and browning become visible. Scald obviously occurs only after a certain amount of breakdown, the complete breakdown of the cell at this stage is probably promoted and accelerated by the presence of a high or toxic concentration of acetaldehyde.

Changes in acidity as presented in Fig. 10 indicate a continuous decrease during storage. In fruit of the various treatments acid reduction occurred in the order (greatest to least) : control, oil wrapper, maize oil emulsion, mineral oil emulsion ; the respective rates of respiration also varied in the same order. As with pears, a high rate of respiration appears to be associated with low acidity. The number of aldehyde determinations was not sufficient to construct complete curves, but the few available figures indicated a similar relationship between changes in acid and aldehyde content.

DISCUSSION.

It is clear from the above results that there is an inverse relationship between the general level of respiration rate as a fruit ages and its juice content. The maintenance of a continued low respiration rate maintains a high level of expressible juice in the fruit for a relatively long time. A low respiration rate signifies low cell activity, slow consumption of the reserves stored in the fruit and therefore longer life for the fruit before final senescence and breakdown. That this is true for untreated fruit has been shown by Smith (1940) who found that varieties of apples having a naturally long storage life are characterized by having large cells and a low respiration rate ; and it is probably true also of cultural treatments which maintain a slow rate of development of the fruit in the orchard, thus no doubt helping to induce a slow rate of respiration. The natural waxy or hairy covering of many fruits is doubtless of great importance in determining the permeability of the epidermis to oxygen, and thus exerting a measure of control over the respiration rate of the fruit. Covering the fruit with an oil film supplements this natural protective layer and opens up new possibilities as a method for controlling the respiration rate.

The action of the oil in depressing the respiration rate is no doubt largely due to its controlling influence on the rate of gas exchange from the external atmosphere to the internal tissues of the fruit. The fruit is covered by a film of diminished porosity, and in some respects the result obtained is very similar to the effect of surrounding the fruit with atmospheres containing increased concentrations of carbon dioxide ; but the oil treatment is a much simpler and less expensive procedure.

There is general agreement in the cycles of change in the respiration rate and juice content as the fruit matures. Thus, at the beginning of the climacteric the juice content and the respiration rate rise together, reaching a peak at about the same time and then declining simultaneously. When the respiration rise is slower, however, the juice content rises to a much higher level ; and with a low and delayed climacteric rise and a slow decline in the respiration rate during the post climacteric stage the juice content is maintained at a high level for a much longer time. The fall in the juice content as the respiration rate declines during the later stages of senescence is no doubt explainable by the breaking down of the cell-cementing material, so that the individual cells are free to move when pressure is applied, with the result that the cell walls do not rupture, and very little juice is set free. The delay of this process with a slowly declining respiration rate no doubt contributes to the maintenance of the high juice content for a long period. Thus, treatments which increase the respiration rate and shorten

the duration of the respiration cycle, such as the removal of the natural waxy covering or damage and injury to the epidermal layer of the fruit, result in a rapid deterioration of the fruit and low juice content. Such harmful treatments are the use of wetting agents and too strong and too long acid dips for the removal of arsenical residues.

Reyneke (1941) has shown that the fruit of the Peregrine peach goes through a dry stage during the ripening process, attaining a juicy and palatable condition only after this stage is passed. This temporary juicelessness, as well as other forms of it, was explained on the basis of a possible lateral cell movement which may take place as the result of a certain relationship between the relative strengths of the cell-cementing material and the cell wall. Lateral movement is promoted when the relative strength of the cell wall exceeds that of the cell cementing material, and vice versa.

The direct relationship which appears to exist between the amount of expressible juice and firmness of Delicious apples—as indicated by the force required to push a plunger into the tissue of the fruit—seems to support the above explanation of conditions of juiciness and juicelessness. The firmness of apples from the different treatments decreased during storage in the same order as the respective expressible juice contents, viz. mineral oil, maize oil, oiled wrappers and control, the last named showing the most rapid drop in both firmness and juice content. The relatively firm apples treated with mineral oil maintained a higher level of juice as compared with the more rapidly softening untreated fruit.

This relationship between firmness of tissue (or force with which cells are held together) and strength of the cell wall is probably not the only factor which determines the juicy or juiceless condition in fruit. The vital processes of the living fruit take place in a complex system of phases, some liquid, such as the cell vacuole, some colloidal gels, as the denser parts of the protoplasm, and some solid, represented by crystalline cell inclusions. During maturation the living substance of the fruit is undergoing continual change and it is in a continual state of flux thus giving rise to changes in the chemical and physical constitution of the fruit. It is possible, therefore, that changes in the juice content are to be attributed, in part at least, to changes in the proportions of the liquid to the colloidal phases in the cell at different stages in the life history of the fruit.

SUMMARY.

1. A series of experiments on Bon Chrétien pears and Golden Delicious apples were carried out with the object of studying the relationship between respiratory activity and the physical condition of fruit, as affected by various factors and treatments. Changes in the physical condition of the fruit were represented by changes in the quantity of expressible juice obtained when pressure was applied to the tissue under standard conditions.

2. A close relationship was found between rate of respiration, juice content, physical condition of the fruit and storage life. Oil applied evenly in a thin film over the surface of the fruit during the pre-climacteric phase reduced the rate of respiration and increased the juice content. Applied after the pre-climacteric phase, it maintained a low respiration rate and a high level of juice.

3. The effectiveness of various oils in reducing the rate of respiration and maintaining a high level of juiciness varied in the order: Arachis, olive, butter, mineral, Soya bean, maize; thus more or less in the order of chemical saturation. The unsaturated non-drying oils were more effective than the more saturated, medium and drying oils.

4. A reduced rate of respiration was accompanied by a reduced rate of acetaldehyde production, reduced acid consumption and a delayed onset of scald development. It is suggested that the development of scald should be ascribed to senescent breakdown which precedes acetaldehyde formation, the latter being a secondary factor which may accentuate or accelerate the development of this disorder.

5. The effect of high temperature and gas storage conditions was such that in poor quality pears kept at 86° F. under conditions of restricted ventilation in which the carbon dioxide given off by the fruit was allowed to accumulate, the rate of respiration was reduced so that only a small climacteric rise became evident, and their juice content increased to a high maximum. These pears, in contrast to those kept at 58° F., became extremely juicy, highly palatable and developed a full yellow attractive colour.

6. The hormone indolylacetic acid, added to oil in low concentrations, appeared slightly to increase the rate of respiration, while a high concentration of hormone and oil significantly reduced it.

7. The effect of orchard and pre-packing treatments was that

(*a*) pears from trees which received oil sprays showed reduced respiratory activity and broke down and scalded less rapidly than those from trees which were sprayed with lead-arsenate or those from unsprayed trees ;

(*b*) the application of wetting agents, or wetting agents together with hydrochloric acid for the removal of arsenical residues, increased the rate of respiration, and reduced the storage life of Bon Chrétien pears considerably.

8. Maintenance of a low respiration rate and retention of a good physical condition appear to be the most important factors associated with increased resistance of fruit to fungus infection.

9. The fact that changes in physical properties and in metabolic activities—as represented by palatability or eating quality, etc., and by rate of respiration—are both reflected by changes in the expressible juice content, naturally suggests the possibility of using this criterion as a means of gauging quality in general.

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THE SUSCEPTIBILITY OF LETTUCE TO MOSAIC VIRUS IN RELATION TO NITROGEN, PHOSPHATE AND WATER SUPPLY

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INTRODUCTION.

The Mosaic disease of lettuce is widespread in market gardens in this country, but is less common under glass. It has been described by Ainsworth and Ogilvie (1939), and since difficulty was sometimes experienced in infecting plants by mechanical inoculation, it was considered that the virus concerned might prove useful for susceptibility studies under varied conditions. An experiment was therefore undertaken to investigate the reaction of lettuce to mechanical inoculation with Mosaic virus, when growing under glass at three levels of nitrogen, three of phosphate and two of water. The fresh weights of the plants at the conclusion of the experiment were used as indices of vegetative growth.

METHODS.

Seed of Cheshunt Early Giant lettuce was sown in boxes on September 1st, 1943, and 288 plants were transferred to 10-inch pots three weeks later when two foliage leaves had developed. The pots had been filled with a mixture of equal parts of sedge peat and calcareous sand, without the addition of any base manures. The experiment was conducted in a cucumber house, 70 ft. by 13 ft., and extended over the autumn months until early December.

Manurial treatments.—Three levels of nitrogen and three of phosphate were applied in all combinations in two equal top-dressings, on September 24th and October 25th respectively. The total rates of manuring were as follows :

Nitrogen	1 N	1·0 gm. dried blood (13·1% N) per pot.
	3 N	3·0 gm. dried blood (13·1% N) per pot.
	9 N	9·0 gm. dried blood (13·1% N) per pot.
Phosphate	1 P	1·0 gm. superphosphate (15-16% P_2O_5) per pot.
	4 P	4·0 gm. superphosphate (15-16% P_2O_5) per pot.
	16 P	16·0 gm. superphosphate (15-16% P_2O_5) per pot.
Potash		All pots received 1·0 gm. sulphate of potash.

Water levels.—Two water levels were maintained for all treatments by varying the frequency of watering. Dates of watering were as follows :

Medium water.	*High water.*
September 21	September 21
September 24	September 24
—	September 29
October 4	October 4
—	October 15
October 26	October 26
—	November 9

When watering, approximately equal amounts of water were applied to all pots.

Design of experiment.—Two plants constituted a plot and each plot was replicated fourfold. There were thus 8 plants in each of 36 treatments, one half of the plants being healthy controls and one half being inoculated with lettuce Mosaic virus.

Inoculation.—All the plants were sprinkled with a little carborundum powder and on October 7th, when four to six foliage leaves had developed, one half of them were wiped with a pad of cotton wool soaked in the freshly extracted juice of Mosaic-infected lettuce leaves mixed with a little 0·5 per cent. sodium sulphite solution, as recommended by Ainsworth and Ogilvie. The other half, i.e. the control plants, were wiped with a pad of cotton wool soaked in distilled water. All the latter remained healthy. Approximately 63 per cent. of the inoculated plants had developed symptoms of the disease after about five weeks.

House conditions.—Up to October 13th the house was heated at night to maintain the air temperature at 55-60° F., but after this date the heating had unfortunately to be discontinued. Night temperatures ranged from 40° to 50° F. until the middle of November when, on two occasions, the temperature fell to 32-34° F. Botrytis infection began to appear at the end of November, and the immature plants were cut and weighed on December 3rd. The house was ventilated during daylight throughout the experiment. A few aphides (*Macrosiphum gei* Koch) were found late in the season, but no plant that had not been inoculated with the virus developed Mosaic symptoms.

ONSET OF DISEASE SYMPTOMS.

Symptoms of disease began to appear 11 days after inoculation. Leaf crinkling and stunting were preceded by slight vein clearing, but only one plant showed slight necrotic spotting. Mottling of the leaf was entirely absent. The leaves had a bitter, unpalatable taste.

Of the 144 inoculated plants, 90 (62·5 per cent.) were showing typical leaf crinkling and stunting by November 12th (Figs. 1 and 2, Plate I). Ainsworth and Ogilvie, working with seedlings all growing in the same kind of soil in 3-inch pots, obtained 80-100 per cent. infection by the method used. The total numbers of plants under each treatment showing Mosaic symptoms 36 days after inoculation are given in Table I. It is evident that there were differences in the susceptibility of plants growing under different manurial and water conditions.

TABLE I.

Number of inoculated lettuce plants showing Mosaic symptoms under different treatments.
(8 *plants in each treatment.*)

Treatment.	Medium water.	High water.
1N, 1P	1	4
4P	5	5
16P	3	6
3N, 1P	2	8
4P	5	7
16P	3	6
9N, 1P	4	3
4P	8	8
16P	6	6

A statistical examination of the data was made according to the methods of Fisher, and the following "z" values (in heavy type where significant) were obtained :

Nitrogen level	.. ..	**0·6963**	5% pt.=0·5994
Phosphate level	.. ..	**1·0586**	1% pt.=0·8423
Water level	.. ..	**1·2024**	1% pt.=1·0116
Interactions :			
Nitrogen × Water ..	..	**0·7755**	5% pt.=0·5994
Nitrogen × Phosphate	..	0·2421	Non-significant.
Phosphate × Water	..	0·0960	Non-significant.
N × P × Water	.. ..	—	Non-significant.

The data for the treatments showing significant effects are shown in Table II and graphically in text Figs. 3 and 4.

TABLE II.

Treatment totals of number of plants showing Mosaic symptoms.

Nitrogen.		Phosphate.		Water.		Nitrogen-Water.		
							Water. Medium.	High.
1N	24	1P	22	Medium	37	1N	9	15
3N	31	4P	38	High	53	3N	10	21
9N	35	16P	30			9N	18	17
Significant differences	8·4		8·4		10·3		5·96	

From the above data it may be concluded that resistance to infection by mechanical inoculation with the lettuce Mosaic virus is associated with low nitrogen, low phosphate and medium water levels in autumn-grown lettuce. Under summer conditions it is uncertain whether the soil conditions prevailing in this experiment would lead to the production of a heavy crop, although under them it was found that healthy lettuce grown from September to December at these levels compared very favourably in fresh weight with plants grown at higher nutrient levels.

FRESH WEIGHT YIELDS.

The fresh weights of the immature lettuces were obtained when the plants were cut on December 3rd. Those of the healthy plants are given in Table III.

TABLE III.

Fresh weight (gm.) of healthy lettuce. (Means of 8 plants.)

Treatment.	Medium water.	High water.
1N, 1P	44·6	36·8
4P	45·0	34·7
16P	35·0	32·4
3N, 1P	35·3	35·5
4P	51·2	37·2
16P	45·1	57·3
9N, 1P	45·7	46·5
4P	47·9	55·0
16P	39·1	57·1

Statistical analysis of the fresh weight data gave the following significant "z" values:

Nitrogen level	**0·9539**	1% pt.=0·8423
Nitrogen × Phosphate ..	**0·5087**	5% pt.=0·4947
Nitrogen × Water	**0·6610**	5% pt.=0·5994
Phosphate × Water ..	**0·6596**	5% pt.=0·5994

The mean values for the nitrogen-water and for the phosphate-water interactions are given in Table IV, together with the values of significant differences.

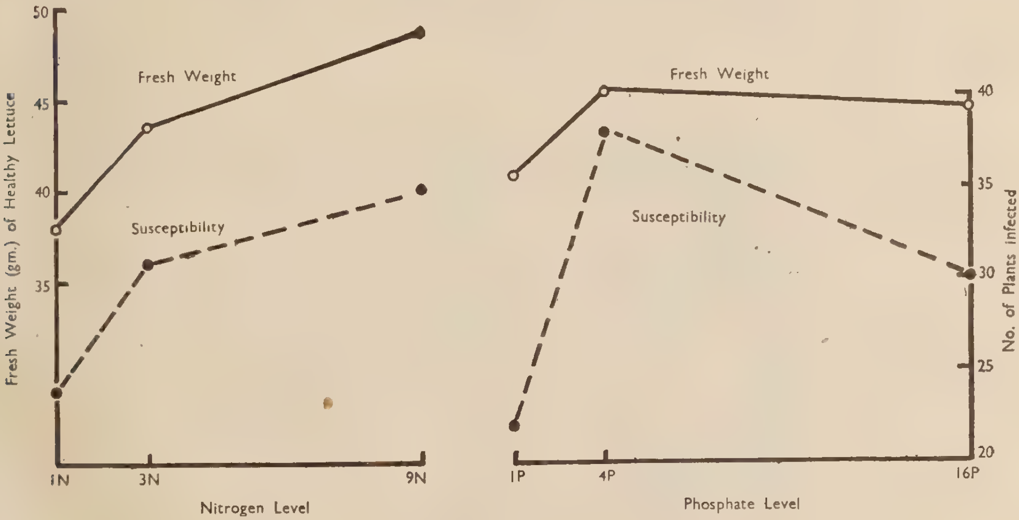

FIG. 3.

Influence of nitrogen and phosphate on growth and susceptibility to Mosaic infection.

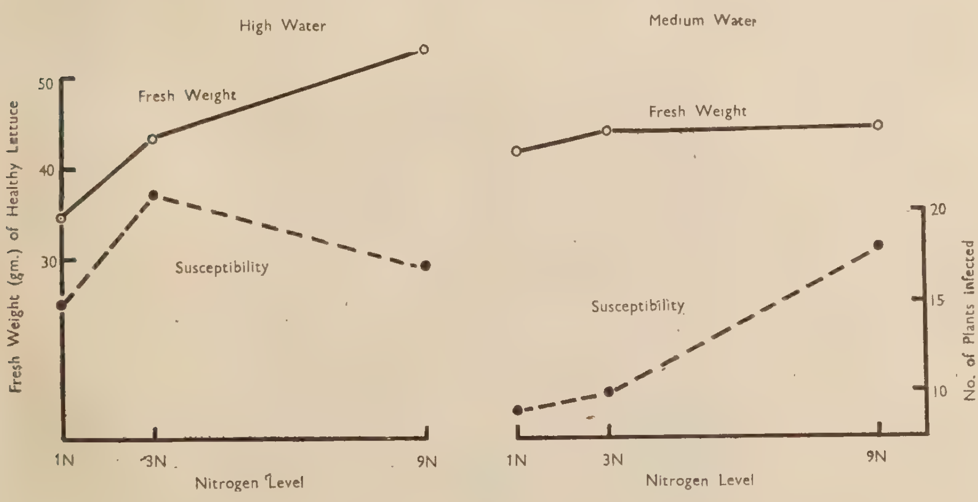

FIG. 4.

Interaction of water and nitrogen on growth and susceptibility to Mosaic infection.

TABLE IV.

Fresh weights (gm.) of healthy lettuce. (Means of 16 plants.)

Treatment.	Medium water.	High water.	Significant difference.
1N	41·5	34·6	8·7
3N	43·8	43·2	
9N	44·2	52·8	
1P	41·8	39·6	8·7
4P	48·0	42·2	
16P	39·8	48·9	

From this Table it will be seen that at a medium water level, increments in either nitrogen or phosphate level failed to induce significant increases in the fresh weights of the plants. At the higher water level, high nitrogen and high phosphate were the only treatments which yielded plants significantly heavier than those grown at low nitrogen or low phosphate at the medium water level.

In Table V are recorded the weights of lettuce infected with Mosaic, together with a comparison of the mean weights of healthy and infected plants. Typical healthy and infected plants at cutting time are illustrated in Fig. 5, Plate I. All the plants inoculated with Mosaic did not become infected, so that a complete statistical analysis of the data could not be made. Three of the infected plants were killed by Botrytis, which was noticeably more severe on the old leaves of infected plants.

TABLE V.

Fresh weight (gm.) of Mosaic-infected lettuce.

Manures.	Medium water.		High water.	
	No. weighed.	Mean weight.	No. weighed.	Mean weight.
1N, 1P	1	21·0	4	8·1
4P	5	20·4	4	12·0
16P	3	4·3	6	9·7
3N, 1P	2	12·5	8	11·9
4P	5	19·0	7	16·1
16P	3	13·0	4	14·5
9N, 1P	4	6·8	3	21·0
4P	8	14·6	8	13·4
16P	6	18·3	6	8·7

Mean weight of 87 infected lettuces=13·6 gm.
Mean weight of 144 healthy lettuces=43·4 gm.
Reduction in fresh weight due to infection with lettuce Mosaic virus=**68·7**%

In text Fig. 6 the interaction between nitrogen and water level for fresh weight of infected plants is illustrated graphically. At a high water level increasing nitrogen led to increased growth of the infected plants and also of the healthy controls (Table IV). At a medium water level, however, nitrogen increments had little influence on fresh weights of either healthy or infected plants, but there is an indication that high nitrogen at this water level may tend to depress the growth of infected plants.

PLATE I.

A B

Fig. 1.

A and B inoculated with lettuce Mosaic virus on October 7th. Symptoms appeared on A on October 23rd. B remained healthy. Treatment: 3N-16P-High water. Photo: November 3rd.

Fig. 2.

Infected plant of the 9N-16P-Medium water series showing the typical leaf crinkling. Necrosis and leaf mottling were not present. Photo: November 3rd.

A B

Fig. 5.

A—Infected lettuce. B—Healthy lettuce.

Photo: December 2nd.

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GROWTH AND SUSCEPTIBILITY TO INFECTION.

At the time of inoculation, no differences in growth or development could be detected in association with the several manurial and water level combinations at which the plants had been growing for about five weeks. Indeed at this time the difference between the water treatments was one watering only. It was therefore surprising to find that water level and the nitrogen-water interaction had significantly influenced the number of plants becoming infected. It seems probable that the range of available nitrogen in the soil over which the plants are resistant to mechanical infection may be quite narrowly defined.

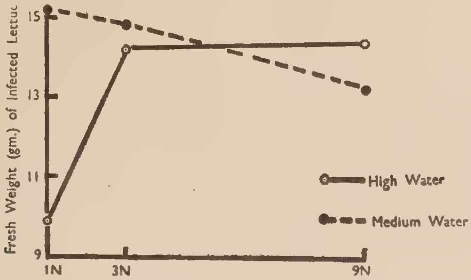

FIG. 6.
Interaction of water and nitrogen on fresh weight of infected lettuce.

In text Fig. 3 the influence of nitrogen and phosphate on susceptibility and final fresh weight of the healthy plants is shown graphically (Means of water treatments). From the nitrogen curves, and to a less extent from the phosphate curves, it appears that susceptibility is related to the growth of the plant. In text Fig. 4, however, in which the nitrogen-water relationship is depicted, it is clear that the growth of the plant may not always bear a simple relation to susceptibility.

SUMMARY.

1. The susceptibility of lettuce to infection by mechanical inoculation with lettuce Mosaic virus has been studied in the variety Cheshunt Early Giant growing in a mixture of peat and sand with all combinations of three levels of nitrogen, three of phosphate and two of watering.

2. Plants receiving high nitrogen and medium phosphate applications were most susceptible (100 per cent. infection). Plants receiving low nitrogen, low phosphate and medium water treatments were most resistant ($12\frac{1}{2}$ per cent. infection).

3. Healthy lettuces grown at the levels of the Mosaic-resistant group did not differ significantly in fresh weight from plants receiving higher levels of nutrients at the same water level. The plants were not grown to maturity, so that no practical conclusion can be drawn from this result.

4. A significant nitrogen-water interaction for susceptibility suggested that the available nitrogen content of the soil may be a factor of importance in determining susceptibility to infection.

5. Infection with lettuce Mosaic virus at the 4-6 leaf stage resulted in a 68 per cent. reduction in fresh weight relative to healthy controls.

ACKNOWLEDGMENT.

Sincere thanks are due to Dr. W. F. Bewley for helpful advice and criticism.

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KEY FOR THE IDENTIFICATION OF THE COMMONLY CULTIVATED COMMERCIAL VARIETIES OF STRAWBERRIES

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CORRECT identification of varieties is an essential requirement in any scheme devised for the certification of true stocks of plants for sale and planting. The fully experienced inspector, with his practised eye, can usually distinguish different varieties at a glance, but it is nevertheless necessary to possess a standard detailed botanical description of each variety to which reference can be made in any doubtful case and to help in the training of new personnel.

Descriptions of strawberry varieties have already been published. Those of Bunyard (1925) cover some 86 varieties, most of them no longer in cultivation ; his interesting notes, however, are more useful for cataloguing purposes than as precise botanical diagnostic descriptions. Maynard's (1929) botanical key of 28 varieties—now mostly out of cultivation—based on the characters of stipules, petiole, and laminar surface has been found difficult to use in practice. Oldham (1937) provided full general descriptions and some illustrations of the then current important commercial varieties, but he did not supply a systematic key for their identification.

The key presented in this paper is confined to ten varieties, but includes all those at present commonly planted commercially in England. It is designed primarily for use in the inspection of runner beds, hence leaf characters alone are used in it. In order to ensure reliable identification the distinguishing characters selected are those which are the most consistent under all conditions. They are : the shape of the leaflet, of its base and of its serrations. Other features which may be more obvious such as vigour, hairiness, leaf pose and (except in extreme cases) colour, have been excluded, since they depend largely on age, conditions of soil and cultivation and are therefore rather variable.

Experience gained by the use of the key may make modifications necessary, and the addition of further varieties may become desirable from time to time. Data for a more comprehensive key, including flower and fruit characters, are being collected. Meanwhile, reports or criticisms from users in various parts of the country as to the value of the key will be appreciated and fully considered by the writers when its improvement and amplification is undertaken.

EXPLANATION OF TERMS USED.

New terms have been made for a number of characters not previously used. Other terms are used with special meanings to adapt them for describing the minute differences which usually exist between varieties. In view of the essentially practical nature of this paper, words in common currency have been used as much as possible. Special care has been taken, however, to define precisely each term used.

Leaflet.—This refers always and solely to the terminal leaflet, i.e. the middle one of the three that normally make up the lamina of a strawberry leaf.

Leaflet shape.—The terms used are (1) " approximately as long as broad ", (2) " slightly longer than broad ", and (3) " much longer than broad ". Inspection of typical examples, as for instance Figs. 6, 10 and 13, will show that these differences are fairly distinct, and with a little experience can be judged at a glance. Still some difficulty may be encountered, as the variability in shape that may occur is by no means negligible.

To settle doubtful cases the following more precise definitions should be used : Category (1) includes leaflets which are slightly broader than long, or only very slightly longer than broad.

If they are usually distinctly longer than broad, this category is ruled out and category (2) or (3) will be required. The division between categories (2) and (3) is based on the ratio $\frac{\text{length leaflet blade}}{\text{breadth leaflet blade}}$ (for short L/B). Category (2) covers leaflets (outside category (1)) in which the L/B ratio is less than 1·2, while category (3) includes leaflets in which the L/B ratio is more than 1·2.

The L/B ratio may conveniently be determined with the help of the graph given in Fig. 1. To do this the lengths and breadths of a few terminal leaflets (after flattening them) should be

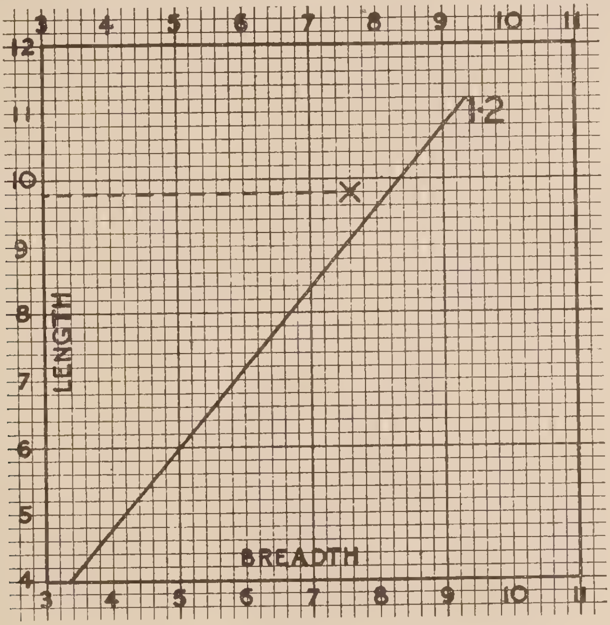

FIG. 1.
Graph for determining leaflet length/breadth ratio.

measured in centimetres and the points corresponding to these measurements located on the graph. For example, suppose the figures found for length and breadth are 9·8 and 7·6 cm. respectively. From 9·8 on the vertical axis, follow horizontally across to the point corresponding to 7·6 on the horizontal axis (dotted line in Fig. 1). The point thus located lies *above* the slanting line representing the ratio 1·2 : 1·0. Therefore the length/breadth ratio in this example is greater than 1·2, and the leaflet would fall into category (3). On the other hand, any point determined from measurements which locate it below the slanting line in the graph will indicate a length/breadth ratio less than 1·2. The graph itself, of course, cannot be used for direct measurement.

Leaflet base angle.—This is the angle formed by straight lines drawn from the tip of the lowest tooth on each side of the base of the terminal leaflet to the point of junction of leaflet and petiole (Fig. 2). The angles are classified as: "slightly acute"—a little less than 90°; "slightly obtuse"—a little more than 90°; "obtuse"—approximately 120°; "very obtuse"—135° and over. The angles are shown in Fig. 3.

Leaflet base shape.—"Wedge-like" means that the edges of the leaflet base from the first serration back to the petiole are practically straight and appear like the two sides of a wedge

or V, as shown in Fig. 11 (Tardive). "Rounded" means that these edges are definitely curved, as shown in Fig. 9 (Royal Sovereign). "Slightly rounded" is intermediate between wedge-like and rounded (Paxton, Fig. 10).

Leaflet surface.—A "cupped" leaflet can easily be distinguished from a "curled" one by flattening it. The margin of a "cupped" leaflet will split when flattened (Fig. 5*c*, Western Queen), while that of a "curled" one will flatten without splitting (Fig. 10, Paxton).

Leaflet serrations.—"Sharp" means that the edges of the teeth are approximately straight (Fig. 11). "Rounded" teeth are as seen in Huxley (Fig. 6). "Shallow" means that the

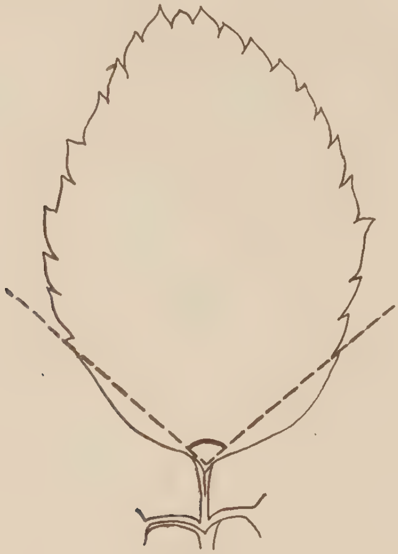

Fig. 2.
Diagram showing leaflet base angle.

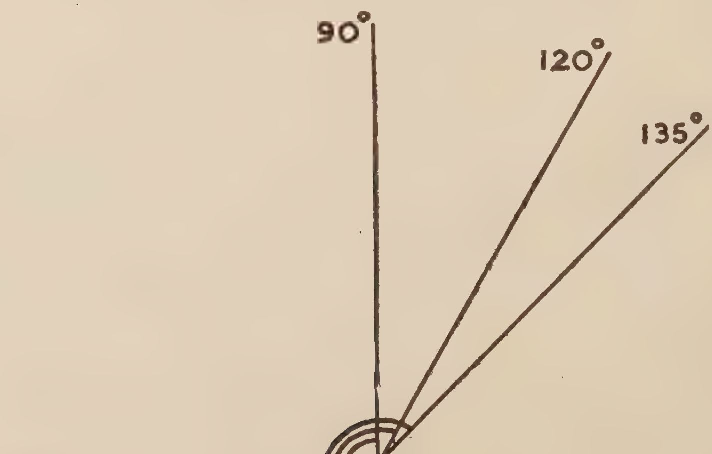

Fig. 3.
Leaflet base angle categories: Slightly acute, a little less than 90°; Slightly obtuse, a little more than 90°; Obtuse, approximately 120°; Very obtuse, 135° and over.

bases of the teeth are as long as their height, "medium depth" that their bases are approximately as long as their height, and "deep" that their bases are shorter than their height. It should be added that vigorous growth tends to round off a sharp serration. Thus allowance may have to be made for vigour.

Hairs on petiole.—"Upward" means that the hairs stand upwards to adpressed against the petiole, or very nearly so. None of the varieties in this key shows this character, but Pillnitz, Little Scarlet, Aberdeen Standard and some others do (Fig. 4*a*). "Outward" hairs make an angle of 90° with the petioles (Fig. 4*b*). On older leaves "outward" hairs sometimes stand in all directions; therefore young petioles should preferably be examined for this character. "Slightly downward" hairs can also best be observed on younger petioles or on the upper part of the petiole, near the leaflet base (Fig. 4*c*).

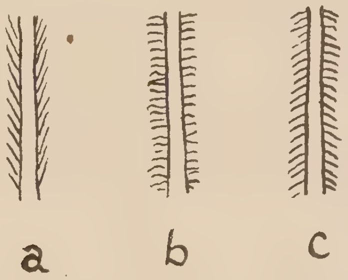

Fig. 4.
Petiole hair categories:
(*a*) upward; (*b*) outward; (*c*) slightly downward.

Petiole furrow.—This refers to the groove sometimes present along the upper side of the petiole. In the key the degree of grooving *on the basal half* of the petiole of fully expanded leaves, of medium age, is the character used.

Seasonal variation.—The characters described above are clearest in summer, which is therefore the best time for beginners to start learning to use the key. Certain characters may change slightly as the season advances, but if due allowance is made for this the key may be used later in the year. For instance, in autumn, strawberry leaflets may become slightly narrower, and the leaf base angle slightly smaller than they are during the summer, probably because of drying out before dying off. Varieties that have a tendency to "cupping" may show this character more markedly in the autumn. The contrast in petiole colour, used to distinguish Royal Sovereign from Paxton, diminishes in autumn, leaving the other characters as the main distinguishing features.

Use of the key.

This is done by fitting the plant under consideration first into one of the main groups A_1, A_2 or A_3 and then step by step into the subdivision which fits its characters. The best way to practise is to work at first with known varieties. In this way the exact meaning of the various descriptions such as "leaflets very much wrinkled", for Mme. Lefebvre, or "coarse rounded serrations" for Huxley, will soon become clear and familiar. Single leaves should never be relied on for identification purposes, since a certain amount of variation is quite common. Several, say five, average fully expanded leaflets of *medium* age should be examined for leaflet shape characters.

Serrations tend to be more rounded on older leaflets. Petiole hairs are best observed on young leaves. Diseased or damaged leaves should, of course, be avoided.

IDENTIFICATION KEY.

A_1 LEAFLET APPROXIMATELY AS LONG AS BROAD.

- B_1 Leaflets very much wrinkled.
  Base angle very obtuse (serrations start low).
  MME. LEFEBVRE (Fig. 6).
- B_2 Leaflets not or slightly wrinkled.
  Base angle (slightly) obtuse.
  Base wedge-like to slightly rounded.
  Serrations fairly sharp and usually coarse and deep.
  THE DUKE (Fig. 12).

A_2 LEAFLET USUALLY SLIGHTLY LONGER THAN BROAD.

- B_1 Serrations coarse and rounded.
  - C_1 Petiole hairs outward.
    Leaflets dark green, shiny, upper part often turned down.
    HUXLEY (Fig. 7). (syn. Brenda Gautrey, Evesham Wonder).
  - C_2 Petiole hairs slightly downward.
    Base slightly rounded.
    Base angle (slightly) obtuse.
    OBERSCHLESIEN (Fig. 8).
- B_2 Serrations more or less sharp.
  - C_1 Leaflets strongly cupped.
    Base (slightly) rounded.
    Base angle often slightly acute.
    WESTERN QUEEN (Fig. 5*b* and *c*).
  - C_2 Leaflets not or slightly cupped.
    - D_1 Base rounded.
      Base angle obtuse.
      Petiole red or at least red-tinged.*
      Petiole, below the middle, not furrowed on leaves of medium age.†
      ROYAL SOVEREIGN (Fig. 9).
    - D_2 Base slightly rounded.
      Base angle (slightly) obtuse.
      Petiole, light green.
      Petiole, below the middle, slightly furrowed on leaves of medium age.†
      SIR JOSEPH PAXTON (Fig. 10).
    - D_3 Base wedge-like.
      Base angle often slightly acute.
      TARDIVE DE LEOPOLD (Fig. 11).

A_3 LEAFLET USUALLY MUCH LONGER THAN BROAD.

- B_1 Base angle often slightly acute.
  Serrations, coarse, often deep and slightly rounded.
  PERLE DE PRAGUE (Fig. 13).
- B_2 Base angle, slightly obtuse.
  Serrations, not coarse, medium depth, often shallow and sharp.
  EARLY CAMBRIDGE (Fig. 14).

* Dark red in maiden plants ; less red or red-tinged in older plants and in maiden plants late in the season.

† This character may be useful in the autumn.

Variety.	Mme. Lefebvre.	The Duke.	Huxley (syn. Brenda Gautrey).	Oberschlesien.*	Western Queen.	Royal Sovereign.	Sir Joseph Paxton.	Tardive de Leopold.	Perle de Prague.	Early Cambridge.
PETIOLE Colour.	Green.	Red-tinged.	Green.	Usually red-tinged.	Usually green.	Dark red in maiden plants, paler red in older ones.	Green.	Usually red-tinged in maiden plants, green in older ones.	Red-tinged.	Red.
Hairs.	Outward.	Outward.	Outward.	Slightly downwards.	Outward.	Outward.	Outward.	Outward.	Outward.	Outward.
LEAFLET. Colour.	Green.	Light sulphur-green with yellow rim.	Dark green, shiny.	Dark green.	Dull green.	Green.	Bright green	Medium green, older leaves dark green.	Green, older leaves dark green.	Green, older leaves dark green.
Hairiness.	Variable.	Hairy.	Hairless.	Hairy.	Hairy.	Few hairs.	Slightly hairy.	Few hairs.	Some hairs.	Hairy.
Shape.	Approx. as long as broad, very much wrinkled.	Approx. as long as broad, not or slightly wrinkled.	Usually slightly longer than broad, often bending over along main vein.	Usually slightly longer than broad.	Usually slightly longer than broad, much cupped.	Usually slightly longer than broad.	Slightly longer than broad.	Usually slightly longer than broad.	Usually much longer than broad.	Usually much longer than broad.
Base.	Slightly rounded.	Wedge-like to slightly rounded.	Slightly rounded.	Slightly rounded.	Rounded.	Rounded.	Slightly rounded.	Wedge-like or nearly so.	Wedge-like.	Wedge-like or nearly so.
Baseangle.	Very obtuse.	Slightly obtuse to obtuse.	Variable.	Slightly obtuse.	Slightly acute to slightly obtuse.	Obtuse.	Slightly obtuse to obtuse.	Slightly acute (in older leaves) to slightly obtuse.	Slightly acute to slightly obtuse.	Slightly obtuse.
Serrations.	Fairly sharp to slightly obtuse, often shallow.	Fairly sharp, usually coarse and deep.	Rounded.	Coarse rounded.	Sharp.	Sharp to slightly rounded.	Sharp to slightly rounded.	Sharp to slightly rounded.	Slightly rounded, medium depth.	Fairly sharp, often shallow.
REMARKS.	—	—	—	Stamens distinctly shorter than receptacle.	—	Petiole, below the middle, not furrowed on leaves of medium age.	Petiole, below the middle, slightly furrowed on leaves of medium age.	Flower imperfect (stamens rudimentary).	Flower petals slightly longer than broad, obovate.	—

* It is considered that the variety described is the true Oberschlesien. There are some others, markedly different, sometimes met with under this name.

Frequently a subdivision is based on two or more characters. The first character, when compared with its counterpart, will generally suffice to establish the division ; but, in view of the close similarities of some varieties, it is important to check also the supplementary characters which are given. When the varietal name which fits the characters is finally reached, it is advisable to compare the plant with the fuller description given in Table I. Reference to the photographs of the leaflets (Figs. 5-11) will also help, both in the earlier and later stages of the determination.

It is as well to stress the limited scope of a key containing a small number of varieties. If the plant examined is in fact one of the varieties included, the key should definitely show *which* one it is. If the variety is not one of those included, the key may establish this fact, but it is just possible that some other variety—especially a new seedling—might conform sufficiently closely to the description of one of the varieties in the key as to be mistaken for it. Checking by comparison with the full description in Table I is therefore most desirable.

ACKNOWLEDGMENTS.

The writers express their gratitude for advice and helpful criticism from, and for facilities provided for examination of material by, Mr. J. M. S. Potter, the Royal Horticultural Society's Fruit Trials Officer ; Mr. C. H. Oldham, of the Ministry of Agriculture and Fisheries ; and Mr. Howard Jones, of the Kingsley Fruit Farms and Nurseries. They also thank Miss E. C. Thompson for taking the photographs and drawing the diagrams. The work was carried out under a special grant from the Agricultural Research Council.

SUMMARY.

An identification key, based on leaf characters and designed especially for use with plants in runner beds, is given for the strawberry varieties Royal Sovereign, Huxley, Sir Joseph Paxton, Tardive de Leopold, Oberschlesien, Mme. Lefebvre, Western Queen, The Duke, Early Cambridge and Perle de Prague. The characters employed are defined and a Table is supplied which gives details of other distinguishing characters of these varieties.

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(*Received* 20/1/44.)

PLATE I.

Fig. 5a.
Leaf of Mme. Lefebvre. Note very much wrinkled surface.

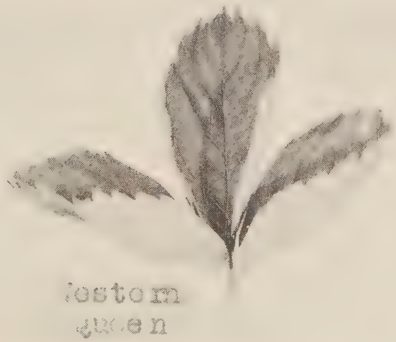

Fig. 5b.
Leaf of Western Queen. Note " cupping ".

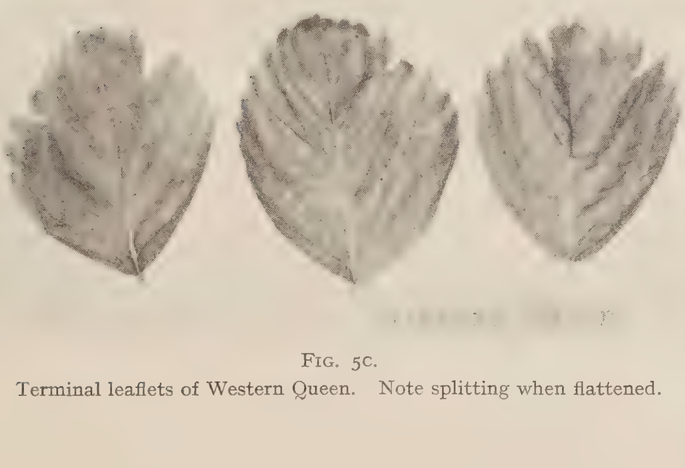

Fig. 5c.
Terminal leaflets of Western Queen. Note splitting when flattened.

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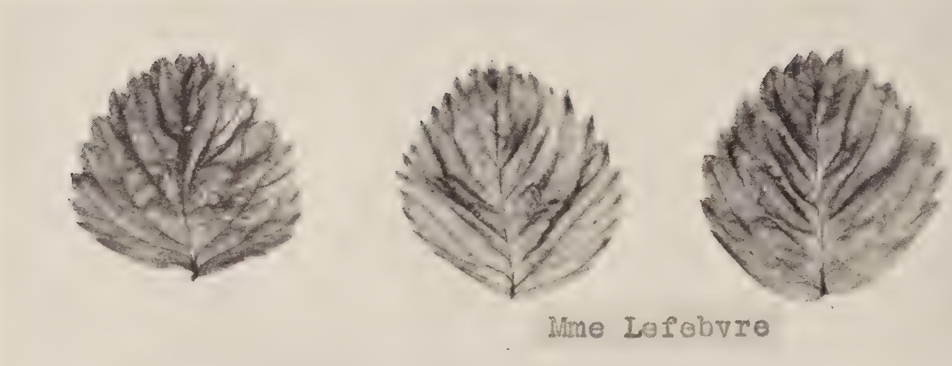

FIG. 6.
Terminal leaflets of Mme. Lefebvre. Note shape approximately as broad as long.

FIG. 7.
Terminal leaflets of Huxley. Note coarse, rounded serrations and variation in base and shape characters.

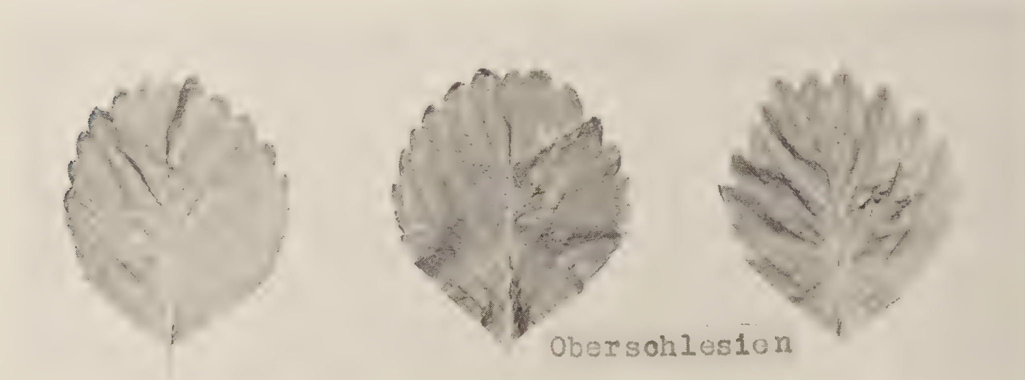

FIG. 8.
Terminal leaflets of Oberschlesien. Note rounded serrations, less so in young leaflet (on right).

FIG. 9.
Terminal leaflets of Royal Sovereign. Note rounded leaflet base.

FIG. 10.
Terminal leaflets of Sir Joseph Paxton. Note slightly rounded leaflet base.

FIG. 11.
Terminal leaflets of Tardive de Leopold. Note wedge-like leaflet base and slightly acute leaflet base angle.

FIG. 12.
Terminal leaflets of The Duke. Note shape, approximately as broad as long.

FIG. 13.
Terminal leaflets of Perle de Prague. Note shape much longer than broad: coarse, often deep and slightly rounded serrations; wedge-like leaflet base and slightly acute leaflet base angle.

FIG. 14.
Terminal leaflets of Early Cambridge. Note shape much longer than broad; sharp and often shallow serrations

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THE INTENSIVE CULTURE OF HARDY FRUIT TREES

I. TRIALS OF COX'S ORANGE PIPPIN AND WORCESTER PEARMAIN APPLE CORDONS

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INTENSIVE methods of fruit culture began to arouse interest about twenty-five years ago. By 1930 many cordon, and a few dwarf pyramid, plantations had become established, and the Station's technical section was receiving enquiries about these forms of tree. Three cordon trials were therefore planned to help to answer some of the problems raised, and they were planted in 1935, 1938 and 1941. The varieties used in all three trials were Cox's Orange Pippin and Worcester Pearmain. These were chosen as differing greatly in habit of growth. The spur-bearing habit of Cox was obviously more suited to the normal methods of intensive culture; but since the tip-bearing variety Worcester was often used as a pollinator for Cox, it was included in order to study the effect of different cultural treatments on growth and cropping and to find out whether it could be grown successfully as a cordon by some modification of the normal culture.

I. A TRIAL OF ROOTSTOCK, SPACING AND PRUNING FOR APPLE CORDONS: RESULTS DURING THE FIRST 10 YEARS.

Maiden (one-year-old) trees budded on rootstocks Malling Nos. I, II, IV, VII, IX and XII were planted in February 1935. The rootstocks varied in vigour from the very dwarf M. IX to the very vigorous M. XII and included two which, as bush trees, have induced vigour at first and heavy crops after about nine years, viz. M. IV and M. VII (Hatton, 1927, 1935, 1939). M. XII was included to discover whether restriction of growth by close planting and summer pruning would make possible the successful intensive culture of trees on very vigorous rootstocks.

Two distances of planting in the rows were used, viz. 1 ft. 9 in. and 3 ft. 6 in. The former is a little closer and the latter wider than the average distance for cordons in most commercial plantations. The rows were always 6 ft. apart. In the absence of experimental data on the effect of summer pruning apple cordons, two methods (illustrated in Diagram 1) were chosen, based on the experiences of commercial growers. In the first all the pruning was carried out in summer, about mid-July and mid-September. At these times mature laterals from the main stems were cut back to about 3 inches, and laterals from spurs to about 1 inch. (The word spur is used to indicate a short branch system, up to about 1 foot in length, arising from the main stem.) Soft, immature laterals were not cut, but were allowed to remain until the next pruning period. It is important to note that the leading shoots, or extension growths, were not cut.

In the second method the trees were pruned in summer, in August, and in winter, about the end of December. During the first week in August all the laterals were cut to 6 or 7 inches. In December, laterals from the main stem were cut back behind the summer pruning cut to 3 inches and those from spurs to 1 inch. The leading shoot of each tree was not cut either in summer or winter.

In the following pages these pruning treatments are referred to respectively as methods S and W: S representing summer and W winter, since all the pruning in the first method was given in summer, while in the second a large part of the pruning was carried out during the dormant period, and only a relatively light one in late summer.

Although the final length of shoot after cutting was the same in both methods, viz. approximately 3 inches, in shoots from the main stem, and 1 inch, in shoots from spurs, by 1943 the

spurs of the trees pruned by method S were longer and more complex than those pruned by method W (see Plate I, Figs. 1 and 2). No doubt this difference was due to the secondary growth which occurred in most years on trees pruned by method S, so that the spurs often increased in length by 2 inches in a single season, viz. 1 inch in July and 1 inch in September. The secondary growth between July and September probably also accounted for the fact that the spurs of the S series were lighter and more delicate in appearance, being composed of a greater number of thinner shoots than those of the W series.

Owing to the nature of the trial it was impossible to give separate cultural treatments to trees of different vigour, and the whole plantation was clean cultivated throughout. Under commercial conditions, however, it would probably have been wise to use a cover crop or a grass mixture for the trees on the more vigorous rootstocks after about 4 years.

Diagram 1.

Diagrammatic representation of the two methods of pruning used in Trial I,—S in July and September and W in August and December.

No bulky manures were applied, since the soil contained plenty of organic matter when the trial began. Six hundredweight of meat and bone meal per acre was given in March in 1938, 1942 and 1943, and small dressings of inorganic manures from time to time as follows :

March	1938	2 cwt. sulphate of potash per acre.
	1940	2 cwt. muriate of potash per acre.
	1941	2 cwt. sulphate of ammonia per acre.
	1942	3 cwt. sulphate of ammonia per acre.

Planning and planting.

This was the first attempt at East Malling, or elsewhere, as far as the writer is aware, to plan a complex trial with cordons, and it presented certain special difficulties which influenced

the design. All three factors, rootstock, spacing and pruning, seemed likely to influence the vigour (and thus the length) of the trees, the first two probably having more influence on vigour than the last one. With wiring of limited height, the angle at which the trees are trained must differ according to the length of tree, unless the extension growth is severely restricted, which is impracticable in single cordons. Cultural and management difficulties would have been increased if individual trees of very different vigour were scattered at random ; and the adoption of unit plots larger than one widely spaced tree, or two closely spaced trees, would have very greatly increased the size of the trial and the labour entailed in carrying it out. Furthermore, placing widely spaced trees adjacent to closely spaced ones would entail the inclusion of many guard trees. The following plan was therefore decided upon (see Diagram 2). There were forty-eight

DIAGRAM 2.

Plan of one Block in Trial I, showing the arrangement of the trees.

combinations of spacing, rootstock, pruning and variety, and these treatments were arranged in eight randomized blocks. Each block consisted of four rows, two of which contained 24 trees each, spaced at 1 ft. 9 in., while the other two contained 12 trees each, spaced at 3 ft. 6 in. The pairs of rows were divided into six randomized plots of eight closely spaced trees each, or four widely spaced trees each, each plot containing trees on one of the six rootstocks included in the trial. These plots were then again divided, half the trees being Cox and half Worcester. Finally, half the trees of each variety were pruned by method S and the rest by method W. Thus in the closely spaced rows the final unit was two trees and in the widely spaced rows one tree. This method is known as a triple split plot design and is a modification of the well-known randomized block method. As will be seen, this method was not wholly satisfactory owing to the

inclusion of treatments producing trees of very different vigour and to the shading of the trees pruned in July by those which were not pruned until August.

The main stems of all the trees were kept quite straight from ground to top. The trees were planted upright with the unions about 3 in. above ground level and were lowered, without bending the stems, to an angle of 45° in August 1935, about six months after planting. The shoot arising from the " bud " was placed on the side opposite to the direction in which the trees were to be lowered, i.e. the maiden shoot was on the north and the trees were trained towards the south. If this precaution is not taken the trees may break when they are lowered to an oblique angle. The trees on the vigorous rootstocks grew so rapidly that they soon reached the top wire, at 6 ft. from ground level, and it was necessary to lower all the trees in alternate years until, in 1943, they were at an angle of 25° to the ground. All the trees in a row had to be lowered at the same time to keep them roughly parallel. By February 1943 the trees in the closely planted rows became too crowded, owing to the acute angle at which they were growing, and alternate plants were then removed.

RECORDS OBTAINED

Before this trial no accurate experiments on cordons had been attempted, and new problems in the measurement of growth and cropping had to be faced.

Vigour.—Both methods of pruning involved the removal of much lateral growth in summer, so that winter measurements of the length of new shoots were impossible. The length of the main stem was, however, measured, and the shoots removed at pruning (including leaves in summer) were counted and weighed. In cordon trees, spurs would normally be encouraged to develop down to within about six inches of the union, but in order to obtain an annual measurement of girth no spurs were allowed to develop below a fixed point, marked with white paint, nine inches above the union. The number of spurs per metre was recorded as a measure of the effect of the treatments on spur development, since it is detrimental in cordons to have considerable lengths of the stem unfurnished with spurs. In 1943 the alternate trees lifted from the closely planted rows in two blocks were weighed. It was not possible to lift the whole root system without damaging the roots of neighbouring trees, so the roots were cut off at the union and only the scion was weighed. This probably gave the best comparison of total vigour between varieties, rootstocks and pruning treatments, though, as will be seen, it did not necessarily show up the differences in character of growth between the trees.

Cropping.—The usual methods of recording fruitfulness at East Malling are by counting the fruit-buds and recording the number and weight of fruit. The former was easy to do on cordons, and annual counts of fruit-buds were made. The recording of all the fruit was made more difficult because of the impossibility of deciding exactly from which tree a fallen fruit had come. Thus, the only way to make sure of a record of total crop was to count the fruit on the trees well before picking time. This was adopted as the main comparative record of fruit, while samples of the picked fruit only were used to measure any differences in fruit size and colour.

In relating fruitfulness to tree size, fruit buds or fruit per unit length of main stem, or per unit area of stem cross section (estimated from the girth), were calculated.

A severe frost occurred in May 1935, a few months after the trees had been planted. In 1938 and 1941, also, almost all the blossoms were destroyed by frost. There were few fruit buds in 1938, but in 1941 the trees blossomed freely, bearing almost as many fruit buds as in 1943; and they would probably have set a good crop if the blossom had not been killed. The extent of the frost damage is shown by the crop comparison for these two years (1941, 1, 1943, 37, fruits per tree). In 1942 the Cox trees bore very little blossom, although they did not crop the previous year.

Statistical analysis of results.—The widely and closely spaced treatments were analysed separately, since the error is not the same with different distances of planting.

In order to equalize the errors for trees on both dwarfing and vigorous rootstocks, which differed widely in size, the numerical data used for the final analyses were transformed to their logarithms (Williams 1937 and Pearce 1943). In consequence the absence of an interaction means that the main effects concerned have had the same proportional effect throughout. Thus the absence of an interaction between pruning and rootstock in the analysis of area of cross-section of trunk means that the decreased vigour due to summer pruning, though greater in absolute amount with the vigorous rootstocks, was proportionately the same with both dwarf and vigorous trees. In the Tables which follow, the transformed values have been omitted for the sake of simplicity and the actual averages of growth or number of fruits, etc., are given. Where the above method of analysis showed differences not significant at the $P=0 \cdot 05$ level, the averages are bracketed. For the closely spaced trees in 1943, after thinning, only the larger differences proved significant. Doubtless there had been competition leading to differences between adjacent trees before thinning. The variation was therefore abnormally large when the plots came to be represented by one tree only instead of by the mean of two.

RESULTS.

1. *Variety.*

(*a*) *Vigour.*—The Cox trees were much longer than the Worcesters (Cox 4·0, Worcester 3·0 m.) and had a greater weight of prunings (Cox 12·7 lb., Worcester 11·7 lb.), but there was no difference in area of cross section of stem, or in weight of scion at lifting, between the two varieties. This was, no doubt, because the shoots of Worcester were thicker (Worcester 67, Cox 83 shoots per lb.). On Worcester, also, the spurs were farther apart than on Cox (Worcester 12, Cox 17 spurs per metre). This effect was largely due to the falling out of fruit buds on the one-year-old leading shoots of Worcester trees, a well-known characteristic of this variety, especially on dwarfing rootstocks, when fruit buds on one-year-old wood are very numerous in the early years. With Cox, although the flowers often failed to set, these fruit buds produced leaves, and in the following year gave rise to spurs.

(*b*) *Fruiting.*—Taking all treatments together the mean number of fruit-buds for Cox was higher than for Worcester, but when the varieties were considered in relation to the pruning treatments it was evident that the Worcesters pruned by method S bore almost as many fruit buds as the Cox trees and actually more fruit buds per unit length of main stem than any of the Cox trees.

The total crop of Cox was about 38 per cent. more than that of Worcester (1935-43, Worcester 63, Cox 102 fruits per tree), but the same pruning effect was evident in cropping as in the production of fruit buds, namely that the Worcesters pruned by method S bore almost as many fruits as the Cox trees and actually more fruits for their size, i.e. per unit length of main stem, than any of the Cox trees.

2. *Rootstock.*

(*a*) *Vigour.*—The cross-sectional area of stem calculated from the girth showed clear-cut differences in vigour due to rootstock effect (see Table I). Trees on all the rootstocks in the

TABLE I.

Cross-sectional area of stem of ten-year-old trees on different rootstocks, 1943 (*sq. cm.*).

Rootstock	IX	VII	II	I	IV	XII
Widely spaced trees	8·0	14·7	17·6	18·1	20·6	26·5
Closely spaced trees	6·9	10·9	13·2	12·3	14·4	20·7

widely spaced rows differed significantly from each other, except on M. I and M. II. The range was somewhat smaller in the closely spaced rows, thinned the previous year, and in these, trees on M. IV were not significantly thicker than those on M. I and M. II.

The annual percentage stem girth increment, a measure of the growth rate, showed that trees on M. IX and M. II were similar in this respect in the early years, and it was much smaller than that of the trees on the other rootstocks. In later years trees on M. II increased in growth rate, and towards the end of the period it was much less in trees on M. IX and greater in those on M. XII than in those on the other four rootstocks.

Differences in length of tree were less than those in girth ; nevertheless, by 1943, trees on M. XII were 27 per cent. longer than those on M. IX. There was little difference in length between trees on M. I, II, IV and VII, but the mean length of trees on these rootstocks was 11 per cent. greater than those on M. IX (see Diagram 3).

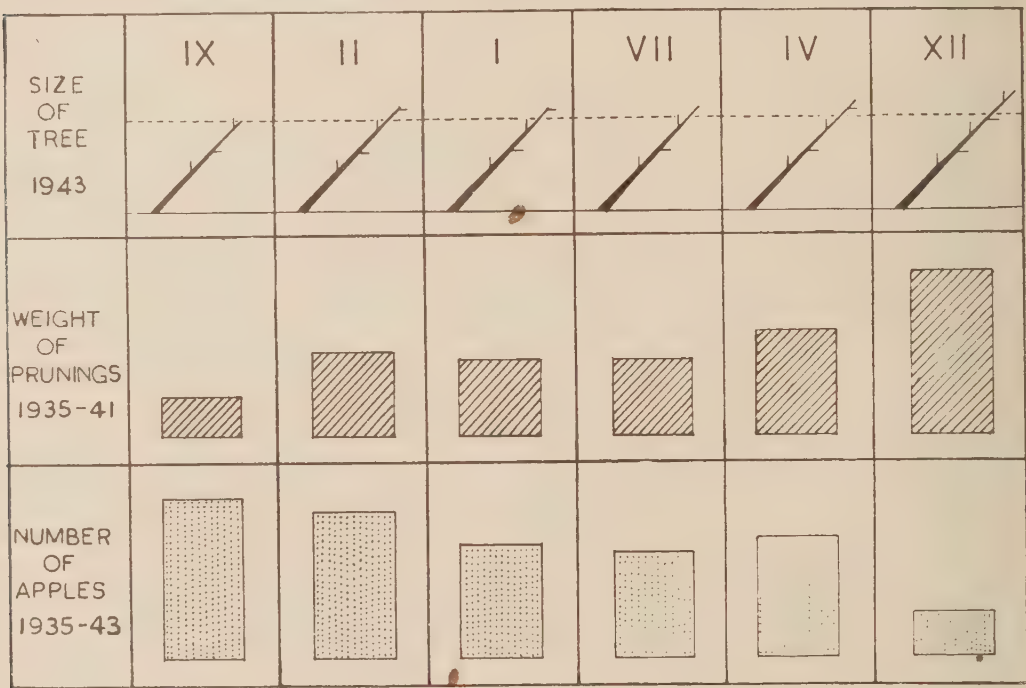

DIAGRAM 3.

Comparison of Rootstock effect on Vigour and Cropping of Apple Cordons.

Trees on M. IX produced only about one half the weight of prunings of those on M. I, II, IV and VII, and about a quarter of those on M. XII (see Diagram 3).

Shoots pruned from trees on M. IX were the smallest and those from trees on M. IV and XII the biggest (M. IV and XII 50, M. IX 64 shoots per lb.).

Of the nine-year-old trees lifted in 1942, those on M. IX were the lightest and those on M. XII the heaviest. There were no differences in weight between the trees on the other rootstocks. Although some of the vigour measurements showed that trees on M. VII were falling off in growth towards the end of the period, the early vigorous growth of trees on this rootstock accounts for their similarity to trees on M. I, II and IV in weight.

The number of spurs per metre was least in trees on M. IX and greatest in those on M. XII (M. IX 13, M. XII 17 spurs per metre). Many of the buds originally present on the one-year-old

eading shoots of trees on dwarfing rootstocks failed to develop into spurs, whereas on vigorous rootstocks a much more even development of laterals was observed.

(*b*) *Fruiting.*—Trees on M. XII had fewer fruit buds than those on other rootstocks although they were well furnished with spurs. In the early years trees on M. IX and II had most fruit buds, especially with Cox ; but in later years they were equalled by those on M. I, II, IV and VII (see Table II).

Table II.

Number of fruit buds per tree on the widely spaced trees on different rootstocks. (1935-42.)

XII	IX	II	VII	IV	I
287	399	401	427	434	442

When the number of fruit buds is considered in relation to tree size, trees on M. IX and II had the greatest number per unit of stem cross-sectional area at the beginning of the period, with those on M. IX considerably ahead of those on M. II. Those on M. IX retained their lead to the end, but, in 1943, those on M. VII were slightly ahead of those on M. II (see Table III). Trees on M. XII were lowest throughout the period.

Table III.

Number of fruit buds per unit area cross-section stem on the widely spaced trees on different rootstocks. (1943.)

XII	IV	I	II	VII	IX
6·3	8·7	10·7	11·1	12·8	16·1

Rootstock differences in cropping are shown in Table IV. Trees on M. XII cropped poorly in relation to the others, while those on M. IX and II cropped heavily.

3. *Planting distance.*

(*a*) *Vigour.*—By 1942 when the closely spaced trees were thinned, the widely spaced trees were 29 per cent. larger in stem cross-sectional area than the closely spaced ones ; they were

Table IV.

Number of fruits per tree on different rootstocks. (1935-42.)

Spacing.	XII	VII	IV	I	II	IX
Close	17	30	34	45	51	65
	XII	IV	I	VII	II	IX
Wide	17	46	52	57	69	78

a little longer (length main stem, close 3·4 m., wide 3·6 m.) and had a much greater weight of prunings (close 9·3 lb., wide 14·9 lb., per tree).

(*b*) *Fruiting*.—The widely spaced trees bore more fruit buds per tree than the closely spaced series (1935-42, close 306, wide 398); they also had a heavier crop per tree (1935-42, close 40, wide 53 fruits per tree). The crop per acre, however, was lower with the widely than with the closely spaced trees (1935-42, close 1,144, wide 611, bushels per acre approx.).

4. *Pruning*.

(*a*) *Vigour*.—The main vigour differences due to pruning, were shown more in the character than in the total amount of the growth (see Plate I). The stem cross-sectional area was greater with pruning W than pruning S (S 16·5 sq. cm., W 18·8 sq. cm.). There was no difference between the pruning methods in regard to length of tree or total weight of prunings. With Worcester, the spurs were a little closer together with method S than with method W (W 11, S 12, spurs per metre).

(*b*) *Fruiting*.—The effects of pruning on fruit bud formation and cropping of the two varieties were not the same. A beneficial effect of pruning by method S on the number of Worcester fruit buds was apparent after five years and increased in amount in later years (1935-42 S 396, W 287, fruit buds per tree), whereas with Cox the number of fruit buds was very little affected by the pruning method.

When these results were being prepared for publication, the 1944 fruit bud records became available and were of such interest that reference will be made to them here, although they do not come into the period under review. The effect was again most marked with Worcester; the number of fruit buds on trees of this variety pruned by method S was much higher than that on trees pruned by method W and higher even than the mean for the Cox trees.

The beneficial effect of pruning method S on Worcester was also shown in cropping. Although the mean crop of Worcester for all treatments from 1935 to 1942 was below that of Cox, Worcester with pruning S bore almost as many fruits as Cox, and actually bore more fruits per unit length of main stem than any of the Cox trees.

The pruning method had less effect on the fruiting of the Cox trees, and the small effect shown was in the reverse direction; that is, they cropped slightly better when pruned by method W. This difference in response to pruning is probably connected with the different habit of growth of the two varieties.

II. APPLE CORDON LEADER TIPPING TRIAL.

A trial of Worcester trees on M. I rootstock was planted in February 1938 to investigate the effect of four varying degrees of leader tipping in the dormant period on single oblique cordons. The trial was planned in four randomized blocks with four trees to a plot. All sixteen plots were arranged in one row with a Cox pollinator between each plot. The trees were 2 ft. apart in the row, which was 6 ft. from adjacent apple cordons.

The trial was designed to find out:

(1) Whether tipping the leaders of cordon Worcesters, which normally form extensive lengths of bare wood when untipped, will induce an even development of laterals along the main stem.

(2) Whether the leader tipping treatments delay cropping unduly.

The treatments were as follows:

(1) Leader not tipped.

(2) One-sixth of the leader removed.

(3) One-third of the leader removed.

(4) One-half of the leader removed.

The laterals were pruned by method W described above.

RESULTS.

The main results of the trial are summarized in Table V. The number of spurs per metre of main stem at the end of 1943, when the trees were seven years old from the bud, was least on the untipped trees and greatest on trees with the most severe leader tipping treatment, viz. one-half removed.

TABLE V.

Growth and cropping of Worcester cordons, 1943.

	Leader treatment—amount removed : None	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$
Length of tree, cm.	198	166	152	117
No. of spurs per m. tree length	8·4	9·8	10·4	11·4
No. of fruits per tree	12·5	11·6	11·4	8·9

In spite of the increase in the number of spurs the crop was least on the trees in which half the leader growth was removed each year. Removing one-third of the leader growth annually resulted in an increase in the number of spurs per metre and only a slight reduction in crop.

Although severe leader tipping had reduced cropping in the early years, the trees in this trial are not yet old enough to show whether leader tipped trees which are better furnished with spurs will ultimately produce more fruit than those left untipped.

III. APPLE CORDON METHOD OF PLANTING TRIAL.

A trial of Cox and Worcester on M. I rootstock was planted in March 1941 in two rows 6 ft. apart with 3 ft. 3 in. between the trees in the rows. The trial included 100 trees, half of which were planted at an angle of 45°, sloping towards the south, and half planted upright. There were eight plots of each treatment, with four Cox and two Worcester trees in each plot. The plots were arranged in pairs, both treatments being represented in each pair.

This was a short-term trial, the object of which was to find out whether trees planted upright and lowered to 45° in the August following planting, as described in Trial I, differ in growth in the first three years from those planted at an angle of 45°.

RESULTS.

In 1944, when the trees were four years old, there was no significant difference in total length of tree or in girth of trunk between the trees planted at an angle of 45° and those planted upright and lowered to an angle of 45° at the end of the summer following planting (see Table VI).

TABLE VI.

Length and girth of main stem of cordons, 1943 (*cm.*).

	Length.		Girth.	
	Upright.	Slanting.	Upright.	Slanting.
Cox	204	197	3·1	2·9
Worcester	140	133	2·8	2·7

As in Trial I, Cox and Worcester differed in length of tree but not in girth of trunk.

DISCUSSION.

From the foregoing results of trials of cordon apples up to ten years old, certain recommendations can be made for the intensive culture of Cox and Worcester, though no doubt these may have to be modified slightly in the future when there has been an opportunity to study the behaviour of the mature trees. The two varieties will be discussed separately, where necessary, since they have responded to some of the treatments in different ways.

Cox has made good cordon trees, well furnished with spurs, on all rootstocks except M. XII. Trees on M. XII were very well furnished with spurs but were over-vigorous for this method of culture and cropped poorly. The trees on M. IX, on the other hand, were particularly easy to prune and manage generally. The amount of wood removed at pruning was small and the work of pruning quickly done. The total crop of Cox trees on M. IX in the first nine years from planting was equal to or above that of trees on the more vigorous M. I, II, IV and VII, showing that these dwarf Cox trees would have been the best investment since they produced an equal, or greater, amount of fruit with less work in pruning.

The method of pruning affected the cropping of Cox very little, although the appearance of the tree was considerably modified. When pruned in July and September (S) the spurs were long and much branched, while when pruned in August and December (W) they were neat and short, with the fruit buds borne close to the main stem.

Worcester made good cordon trees on M. I, II, IV and VII when pruned in the summer only (S), but was not wholly satisfactory on M. IX even with this method of pruning, because the trees were not well furnished with spurs. With intensive systems of culture the aim is to obtain the maximum productivity in the minimum space, consequently extensive bare lengths of main stem are to be avoided. In general, the spurs were much farther apart on Worcester than on Cox, and this characteristic was accentuated in trees on M. IX. It appears that on dwarfing rootstocks many buds on the main stem remain dormant or form fruit buds which fall off, whereas when worked on the more vigorous rootstocks a greater proportion of such buds grow into lateral shoots. With a variety like Worcester, where the spurs normally tend to be rather far apart, it is particularly important to choose a rootstock which induces an even development of lateral shoots from the main stem. Worcester trees on M. IX and II bore the heaviest total crop, but those on M. IX may fall behind in cropping owing to the poor development of spurs as the trees become more mature. M. XII could not be recommended for cordons, even with Worcester ; but Worcester did relatively better than Cox on this rootstock, probably because the trees were better furnished with spurs than when on other rootstocks.

The development of spurs on the Worcester trees was influenced by the pruning method as well as by rootstock, the spurs being closer together when pruned entirely in summer in mid-July and mid-September (S), than when pruned lightly in early August and more severely in late December (W). This may account partly for the fact that Worcester cropped much better when all the pruning was done in summer, though the increased yield may also be due to the effect on fruit bud formation of severe cutting in early summer. Worcester trees, pruned in summer only, bore almost as much fruit as Cox, but the Worcesters pruned in August and December bore little more than half the amount of fruit borne by similarly pruned Cox trees. In 1944, the Worcester trees pruned in summer only, actually bore far more fruit buds than any of the Cox trees. Thus it appears that the choice of rootstock and pruning method are particularly important for cordon trees of a tip-bearing variety such as Worcester, while the spur-bearing Cox may do almost equally well under a wider range of treatments.

It is probable that a successful cordon plantation could be made by planting alternate rows of Cox and Worcester (or two rows of Cox and one of Worcester) with Cox on M. IX or M. II rootstocks and the Worcester on M. I or M. II, the Cox trees to be pruned in summer and in winter (method W) and the Worcesters in summer only (S). If it were desired to use one pruning method, it is suggested that the summer only method should be used, since this suited

Cox quite well and was much the better of the two methods for Worcester. There would be certain advantages, however, in using both pruning methods, because the labour could be spaced out better. It is difficult to provide enough suitable labour to prune a large plantation within a few days about the middle of July.

Both varieties bore more fruit per tree with wide spacing (3 ft. 6 in.) and more fruit per acre with close spacing (1 ft. 9 in.), but the trees became difficult to manage in the closely spaced rows, and it is suggested that trees on M. IX should be planted at 2 ft., on M. I, II and VII at 2 ft. 6 in. and on M. IV at 3 ft. apart on average fertile soils.

Trial III has shown that cordons may be planted at an angle of 45° without any detrimental effect on growth in the first three years after planting, and this has certain advantages as against planting upright, because training in future years is easier and the risk of breaking the main stem is avoided.

For private gardens, where cordon trees may remain for many years, and appearance and space effectively occupied are important considerations, it is recommended that the leading shoots of varieties similar to Worcester in habit of growth and cropping should be tipped, about one-third of the shoot being removed annually to encourage the even development of spurs. There is no need to tip the leaders of Cox cordons, even in gardens, since normal trees will be well furnished without tipping, and tipping will unnecessarily delay cropping.

SUMMARY.

A description is given of three trials of Cox's Orange Pippin and Worcester Pearmain apples grown as single oblique cordons.

In Trial I the treatments were (1) rootstock (Malling I, II, IV, VII, IX and XII), (2) distance apart of planting in the rows (1 ft. 9 in. and 3 ft. 6 in.), and (3) pruning, (i) pruned entirely in summer in mid-July and mid-September, method S, and (ii) pruned lightly in early August and more severely in late December, method W.

In Trial II four leader tipping treatments were carried out on trees on M. I rootstock.

In Trial III the effect of two planting methods was compared : (i) trees planted at an angle of 45°, and (ii) trees planted upright and lowered to 45° the following August.

Trial I.

The results up to the time when the trees were 10 years old were as follows :

(i) *Variety*.—The Cox trees were longer than the Worcesters and had a greater weight of prunings, but there was no difference in stem cross-sectional area, or in weight of scion at lifting. The spurs were much farther apart on the Worcester than on the Cox trees.

When all the treatments were considered together the Cox trees had more fruit buds and more fruit than the Worcesters, but when the pruning treatments were statistically examined separately it was shown that Worcesters with pruning S bore more fruit for their size than any of the Cox trees.

(ii) *Rootstock*.—Trees on M. IX were the smallest in stem cross-sectional area, those on M. VII were next in order of vigour, then came those on M. I and II, which did not differ from each other, followed by those on M. IV and finally those on M. XII. Similar differences in vigour were shown in length of tree and weight of prunings. The weight of prunings from trees on M. IX was only about a quarter of that from trees on M. XII. The weight of scion from the lifted series showed that trees on M. IX were lightest and those on M. XII heaviest. There was no difference in weight between the trees on other rootstocks.

Trees on M. IX and II bore most fruit buds and fruit in the early years, but at the end of the period those on M. I, IV and VII equalled those on M. IX and M. II in fruit buds, though

not in crop. Trees on M. IX retained their lead to the end of the period in number of fruit buds in relation to tree size, i.e. number of fruit buds per unit stem cross-sectional area.

(iii) *Planting distance.*—The closely spaced trees were much smaller than the widely spaced trees. The widely spaced series bore more fruit buds and a heavier crop per tree, but the crop per acre was highest with close spacing.

(iv) *Pruning.*—Trees pruned by method S were slightly smaller and the spurs on them were a little closer together than on trees pruned by method W. Series S trees bore more fruit buds and more fruit than series W with Worcester, but not with Cox.

TRIAL II.

The spurs were closer together on the leader pruned Worcester trees than on those of which the leader was left uncut. The crop in the first six years after planting was greatest on the trees of which the leader was not pruned.

TRIAL III.

Two series of Cox and Worcester cordons (i) planted at an angle of 45° and (ii) planted upright and lowered to an angle of 45° the following August, did not differ in the length or girth of the main stem three years after planting.

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The writer wishes to acknowledge the advice of Dr. R. G. Hatton under whose direction these studies were made. She also wishes to thank Mr. T. N. Hoblyn, for much helpful criticism and advice in the presentation of the results ; Mr. S. C. Pearce, for calculating the analyses of variance ; Miss A. Searle, for help with computing ; Miss E. C. Thompson, for making the diagrams and photographs, and several members of the Pomology Section for recording tree growth and fruiting.

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PLATE I.

FIG. 1.

Worcester Pearmain on M. IX. Note spurs longer and closer together on tree pruned by method S.

FIG. 2.

Cox's Orange Pippin on M. IV. Note spurs longer on tree pruned by method S.

[face p. 52

SOME OBSERVATIONS ON THE RIPENING OF PLUMS BY ETHYLENE

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THE effect of acetylene in stimulating the ripening of South African Kelsey plums was described in 1938 by Putterill (1). Similar results were obtained in this country (Smith, 2) with Monarch plums picked at a relatively immature stage and treated with acetylene in a 1 per cent. concentration, for 48 hours, at 65° F. Further experiments have now been made with the varieties Myrobalan, River's Early, Czar and Monarch, using ethylene instead of acetylene.

RESULTS WITH DIFFERENT VARIETIES.

The results obtained with these four varieties are summarized below:

Myrobalan.—When picked very green and immature (6/7/40), and treated immediately with ethylene at concentrations of 1/200 or 1/500, for 24 and 48 hours, the plums became scarlet-coloured, yellow-fleshed and juicy, and developed the typical ripe aroma in about 9 days at 65° F. The ripened fruits remained acid and did not sweeten. A control sample held at 68° F. was still green after 11 days.

Other samples were similarly treated (17/7/40) after further maturation on the tree. Softening, and development of flavour, aroma and juiciness, was greatly accelerated, as compared with controls held at the same temperature. The colour change was not accelerated to the same degree as the other changes, with the result that the plums had assumed only a somewhat dull red colour by the time they were soft, juicy and sweet.

River's Early.—Plums picked (20/7/40) with less than three-quarters of their surface coloured purple were treated with ethylene at a concentration of 1/1,000. There was accelerated development of colour, earlier softening and the development of stronger aroma than in untreated controls at the same temperature.

Czar.—Plums picked (30/7/40) from one quarter to almost fully coloured (purple) were treated with ethylene (1/1,000) for 24 hours and 48 hours. The more backward fruits showed accelerated development of colour. After three days from picking, hardness, as measured by the mechanical pressure-tester,* had fallen from 18 lb. to 8 lb. (24 hours treatment) and to $7\frac{1}{2}$ lb. (48 hours treatment), as compared with 9 lb. in the untreated samples. The treated samples had a more marked aroma.

Monarch.—The behaviour of the Monarch variety was similar to that of Czar and is described more fully in the next paragraph.

EFFECT OF CONCENTRATION OF ETHYLENE AND DURATION OF TREATMENT.

An experiment was carried out to determine the effect of the concentration of ethylene and of the duration of treatment, using Monarch plums. Ethylene was passed into an air stream flowing at the rate of approximately 10 litres per hour through desiccators containing the fruit, so as to give approximately the following average concentrations: 1/50; 1/250; 1/1,250; 1/6,250; 1/31,000; 1/156,000. In addition, a sample was treated with air which had passed over ripe plums of the variety Giant Prune. Control samples were kept:

(i) in a separate building;
(ii) exposed to the air in the room in which the ethylene treatment was given; and
(iii) in a desiccator through which fresh air was passed from outside the room.

* Diameter of plunger 11 mm.; depth of penetration 0·9 mm.

Each treatment was given for two periods, namely 24 and 48 hours respectively, and the treatment with ethylene at 1/1,250 was also given for 72 hours. The temperature of treatment was 65° F., and the plums were subsequently kept at the same temperature for observation. The chemical samples referred to below were frozen (at 14° F.) on the third and fifth days after the picking date (28/9/40).

Table I indicates the rate of change of colour from green to purple, assessed in terms of the area coloured purple. The numbers given are the percentages of plums over three-quarters coloured. At the time of picking all were under three-quarters coloured. About 25 per cent. were in fact less than one-quarter coloured.

TABLE I.

The development of purple colour in Monarch plums treated with ethylene at 65° F.

Treatment.	Percentage of plums > ¾ coloured. Days after picking. 2	3	5
Control (i)	40	64	100
Control (ii)	—	87	100
Control (iii)	—	60	100
Ethylene 1/50 : 24 hr.	—	89	100
1/50 : 48 hr.	90	100	100
1/250 : 24 hr.	—	74	84
1/250 : 48 hr.	47	63	83
1/1,250 : 24 hr.	—	85	100
1/1,250 : 48 hr.	97	100	—
1/1,250 : 72 hr.	—	—	100
1/6,250 : 24 hr.	—	80	100
1/6,250 : 48 hr.	90	100	100
1/31,000 : 24 hr.	—	86	100
1/31,000 : 48 hr.	44	59	88
1/156,000 : 48 hr.	82	100	100
Air from ripe plums	96	100	100

After 48 hours' treatment all the treated plums were more fully coloured than the controls. The greatest increase in colour was shown by the samples treated with ethylene (48 hours) at 1 1,250, or with air which had previously been passed over ripe plums. The plums treated with ethylene at 1 250 (48 hours) and 1/31,000 (48 hours) showed little advance of colour.

On the third day from picking (after a further day in air), the sample treated with ethylene at 1/250 (both 24 and 48 hours) still showed less advance over the controls than the other samples. The sample treated with ethylene at 1/31,000 for 48 hours was also less advanced. This difference persisted to the fifth day from picking and suggests that the effect of ethylene in concentrations of 1/250 and 1/31,000 may be to inhibit colour change after a certain stage is reached. Samples treated with these concentrations were abnormal in appearance, the uncoloured areas being clearly demarcated and subsequently turning yellow instead of purple. By the fifth day the samples from all treatments except these two, and including the controls, were fully coloured.

In Table II is shown the degree of softening on the third and fifth days after picking as measured by a mechanical pressure-tester. The tester gave a reading of 21 lb. on the freshly picked plums.

Softening was accelerated in all the treated samples as compared with the controls. No divergent effect upon softening was evident in the samples treated with ethylene at 1/250 and 1 31,000. No significant difference in the degree of softening was observed as between treatments with different concentrations of ethylene. The "ripe plum air" achieved the same

TABLE II.

Softening of Monarch plums in relation to treatment with ethylene and ripe plum air.

Treatment.	Pounds pressure. Days after picking. 3	5
Control (i)	19·5	12
Control (ii)	—	13
Control (iii)	17	15
Ethylene 1/50 : 24 hr.	11	8·5
1/50 : 48 hr.	—	8
1/250 : 24 hr.	—	7
1/250 : 48 hr.	—	7·5
1/1,250 : 24 hr.	—	9·5
1/1,250 : 48 hr.	—	7·5
1/1,250 : 72 hr.	—	6·5
1/6,250 : 24 hr.	12	10
1/6,250 : 48 hr.	—	8
1/31,000 : 24 hr.	—	8·5
1/31,000 : 48 hr.	—	8
1/156,000 : 48 hr.	—	7·5
Air from ripe plums	—	7·5

effect as the ethylene. The average test value on the fifth day for the control samples was 13 lb., and for the treated samples 8 lb. Samples treated for 48 hours were slightly softer than those treated for 24 hours. The sample treated with ethylene at 1/1,250 for 72 hours was softer than that treated with the same concentration for 48 hours.

On the fifth day the untreated control samples had scarcely any "ripe plum" odour. All the treated samples had a typical ripe smell.

Flavour was normal except in the plums treated with ethylene at 1/50. In these a rather flat taste was evident when the fruit was cooked.

EFFECT OF ETHYLENE TREATMENT ON SUGAR AND ACID CONTENT.

Table III gives the acid and sugar contents of both treated and untreated plums. The sugar was estimated as total invert sugar and the acid as malic acid.

TABLE III.

The effect of ethylene treatment at 65° F. upon the sugar and acid content of Monarch plums.

Days from picking.	Treatment.	Acid % fresh weight.	Total sugar % fresh weight.
0	Initial (untreated)	1·73	6·30
3	Control on open tray	1·68	7·34
	Control in desiccator	1·43	7·03
	Ethylene at 1/50 : 24 hr.	1·49	7·98
	Ethylene at 1/1,250 : 24 hr.	1·61	7·02
5	Control on open tray	1·65	7·10
	Control in desiccator	1·60	—
	Ethylene at 1/1,250 : 24 hr.	1·58	—
	Ethylene at 1/1,250 : 72 hr.	1·57	7·45

It will be seen that treatment with ethylene had no appreciable effect upon the acid and sugar contents. Work subsequently carried out by Adam and Gillespey (3) is in agreement with this finding.

Conclusions.

The most marked effect of ethylene was to accelerate the softening of the plum. At 65° F. softening was accelerated both by the administration for one and two days either of heavy dosages (1/50) or of slight traces (1/156,000) of ethylene. The effect upon colour change appeared complex, abnormal colour change being produced by certain intermediate concentrations (1/250 and 1/31,000). The effect upon the development of odour and flavour was very marked and was brought about by a wide range of concentrations. No evidence has been found of any appreciable effect of the treatment on the sugar and acid contents of the fruit.

SUMMARY.

Experiments are reported in which plums were treated in air with various concentrations of ethylene ranging from 1/250 to 1/156,000.

The main effect of ethylene was to accelerate change of colour, softening and the development of the typical "ripe-plum" odour. No appreciable effect upon the change in sugar and acid contents during ripening was observed in the small range of samples tested.

Abnormal colour change was produced by ethylene at concentrations 1/250 and 1/31,000.

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POLLEN TUBE GROWTH AND EMBRYO-SAC DEVELOPMENT IN APPLES AND PEARS*

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I. APPLES.

Introduction.

Self-incompatibility in plants is a physiological mechanism to ensure cross-fertilization. It is brought about by inhibition of the growth of the pollen tubes owing to a reaction between the haploid pollen tube and the diploid stylar and ovarian tissue. A large proportion of our cultivated fruits are self-incompatible, and since in most varieties fruit formation is dependent on fertilization and seed development, incompatibility gives rise to a number of practical problems, especially in modern orchards in which it is usual to grow few varieties but a large number of each.

In cultivated apples self-incompatibility grades almost imperceptibly from a few varieties which, under ideal conditions, can set a small crop of fruit with their own pollen, to complete self-incompatibility (Crane and Lawrence, 1938).

Cross-incompatibility in apples is rare, but it is steadily increasing, owing to the introduction of new varieties which are closely related; and this must be taken into account when interplanting such varieties. New varieties which have arisen as bud sports without a change in chromosome number are invariably incompatible with the original variety, and closely related seedlings are often partially incompatible *inter se* and with their parents. This is mainly reflected in the proportion of seeds formed, although under unfavourable conditions it may lead to a reduced crop of fruit.

Incompatibility in apples is usually measured by the percentage of matured fruits to pollinated flowers. This, however, does not take into account the difference between varieties which are entirely dependent upon fertilization for fruit formation, and those which can produce seedless, parthenocarpic fruits. Therefore, unless the seed contents of the fruits are known, results based on fruit set alone can be misleading. Furthermore, it has been shown by a number of investigators that the number of seeds is higher in mature fruits than in those which, owing to nutritional and other causes, drop from the tree before maturity (Kobel, 1931; Brittain and Eidt, 1933). In order to obtain a complete knowledge of compatibility relations, therefore, seed counts should be made early, before the so-called June drop, and the number of seeds plotted against the number of ovules. This basis of estimation must be applied with caution to triploid varieties, because their gametic sterility may obscure the results.

In view of the relatively large number of triploid varieties under cultivation, this sterility is another important factor in fruit setting. It is more pronounced on the male than on the female side. For this reason the pollen of triploids is of little value for other varieties, but triploids pollinated by diploids can set a normal crop.

In practice the effect of partial incompatibility and sterility can largely be reduced by the internal conditions of the tree and by the environment. A healthy tree under normal conditions produces such a large number of flowers that, even if only 5 per cent. of them set fruit, the crop may be good.

Temperature is an environmental condition which has been found greatly to affect incompatibility and pollen tube growth in other kinds of plants. Thus a high temperature, by accelerating the reaction which causes incompatibility, accentuates the difference between

* Part of a Thesis approved for the Degree of Ph.D. in the University of London.

incompatible and compatible pollen tube growth (Lewis, 1942 *a*). It is therefore a useful aid to the interpretation of pollen tube growth results, and it may in some cases affect fruit setting.

In my investigations, therefore, I have made a detailed study of pollen tube growth and embryo-sac development in different self- and cross-pollinations in diploids and triploids at different temperatures. I also had access to the final results of extensive pollination experiments carried out at the John Innes Horticultural Institution, and analysed them in connection with my studies. It is clear that a study of the processes leading to fruit formation is necessary for an elucidation of the problems to which the setting and development of fruit give rise.

Finally, the breeding of self-compatible varieties with their obvious advantages is a possibility, and it is hoped that the present studies may serve as a guide to the selection of the best parents for such work.

Material and methods.

The material used in the investigations was as follows :

Diploids.	*Triploids.*
Baumann's Reinette	Blenheim Orange
Cox's Orange Pippin	Bramley's Seedling
Northern Spy	
N.789 (Northern Spy × M. II)	
Early Victoria	
Lane's Prince Albert	

It was collected from trees grown in pots in a cool glasshouse. Flowers for early examinations, ten and forty-eight hours after pollination, were taken from the trees and incubated at 10° C., 15° C., 20° C., 25° C. and 30° C. Flowers for later examinations, after six and twelve days, were left on the trees for these periods. The age of the flower in apples, after it becomes receptive, has little effect on pollen tube growth, but the precaution of taking flowers at a comparable stage, as judged by the condition of the stigmas, was always observed. The material for studying pollen tube growth was fixed in three parts of absolute alcohol and one part glacial acetic acid, and that for embryo-sac development in either 2 BX (La Cour) or Navashin's fluid after a short treatment in Carnoy.

Before dissection the styles were placed in water for a minute to soften the tissue. After being separated, each style was cut in half longitudinally. The stains used for studying pollen tube growth were either (1) light green/acid fuchsin (2 c.cm. 1 per cent. light green aq., 2 c.cm. 1 per cent. acid fuchsin aq., 10 c.cm. glycerine, 40 c.cm. lactic acid and 46 c.cm. distilled water ; cf. Cummings (1936) *et al.*), or (2) 1 per cent. cotton blue (in lactic acid, phenol, glycerine and water in equal parts). For temporary preparations the sections were mounted in the stain.

Ovaries were embedded in paraffin wax and cut for the examination of the embryo-sacs. The sections, 16μ-22μ thick, were stained in 0·5 per cent. Heidenhain's iron hæmatoxylin.

The lengths of all the pollen tubes in each style were measured, in order to obtain their distribution, but only those of the longest tubes are summarized in the accompanying tables.

Self-incompatibility in apples.

(*a*) *Self-incompatibility in diploids.*—Two diploid varieties were chosen for examination, viz. Cox's Orange, which is highly self-incompatible, and Baumann's Reinette, which is partially self-compatible (see Table I).

The percentage of good pollen is high in these as in other diploid varieties (Wanscher, 1939), i.e. 86 per cent. in Cox's Orange and 97 per cent. in Baumann's Reinette. According to Florin (1927) the pollen germination of these varieties in an artificial medium is 70 per cent. to 100 per

TABLE I.

Setting of fruits in apples. (Data from the J.I.H.I.)

Variety.	No. of flowers.	Fruit set.	
		No.	%
SELFING :			
Diploids :			
Cox's Orange Pippin	12,680	96	0·7
Northern Spy	3,385	24	0·7
Lane's Prince Albert	3,699	57	1·5
N.789	104	2	1·9
Baumann's Reinette	226	17	7·5
Triploids :			
Blenheim Orange	2,110	27	1·3
Bramley's Seedling	1,269	61	4·8
CROSSING :			
Diploids × diploids :			
Cox's Orange Pippin × Lane's Prince Albert	198	20	10·1
Cox's Orange Pippin × Baumann's Reinette	88	4	4·5
Lane's Prince Albert × Cox's Orange Pippin	711	41	5·8
Baumann's Reinette × Cox's Orange Pippin	115	3	2·6
Triploids × diploids :			
Blenheim Orange × Cox's Orange Pippin	167	11	6·5
Bramley's Seedling × Cox's Orange Pippin	148	13	8·8
Diploids × triploids :			
Cox's Orange Pippin × Blenheim Orange	355	24	6·8
Cox's Orange Pippin × Bramley's Seedling	207	14	6·7
Triploids × triploids :			
Blenheim Orange × Bramley's Seedling	65	3	4·6
Bramley's Seedling × Blenheim Orange	35	3	8·6

TABLE II.

Pollen tube growth ten hours after self-pollination.

Variety.	Temperature.				
	10° C. mm.	15° C. mm.	20° C. mm.	25° C. mm.	30° C. mm.
Diploids :					
Cox's Orange Pippin	1·6 (8)	3·9 (8)	4·5 (8)	7·9 (4)	8·3 (8)
Northern Spy	1·2 (5)	2·5 (4)	3·1 (5)	4·0 (10)	5·0 (9)
Lane's Prince Albert	1·9 (5)	2·9 (8)	3·2 (8)	4·0 (9)	4·4 (6)
N.789	1·3 (8)	3·2 (6)	3·9 (8)	4·6 (10)	4·3 (11)
Early Victoria	1·4 (3)	3·6 (4)	4·0 (3)	— —	5·9 (4)
Baumann's Reinette	1·0 (3)	1·9 (9)	3·0 (6)	4·4 (6)	5·0 (6)
Mean (excluding Cox's)	1·4	2·8	3·4	4·2	4·9
Triploids :					
Blenheim Orange	0·5 (5)	1·4 (7)	2·0 (8)	4·0 (9)	3·5 (6)
Bramley's Seedling	0·9 (5)	1·7 (5)	2·8 (3)	4·3 (6)	5·5 (5)
Mean	0·7	1·5	2·4	4·1	4·5

(Figures in brackets indicate the number of styles examined.)

cent. In the present studies, germination of the pollen grains on their own stigmas was found to be good at all temperatures, although consistently lower at 10° C. than at 15° C.-30° C.

Two types of pollen tube were observed in the styles of selfed Cox's Orange and Baumann's Reinette: (1) *fully incompatible tubes* which did not reach the ovary, (2) *semi-compatible tubes* which reached the ovary but seldom effected fertilization. The great majority of the pollen tubes were of the first type, but they could not clearly be distinguished from the semi-compatible tubes until a fairly late period, i.e. when the semi-compatibles had entered the ovary (Fig. 1). At this time (forty-eight hours after pollination) many of the incompatible tubes had swollen

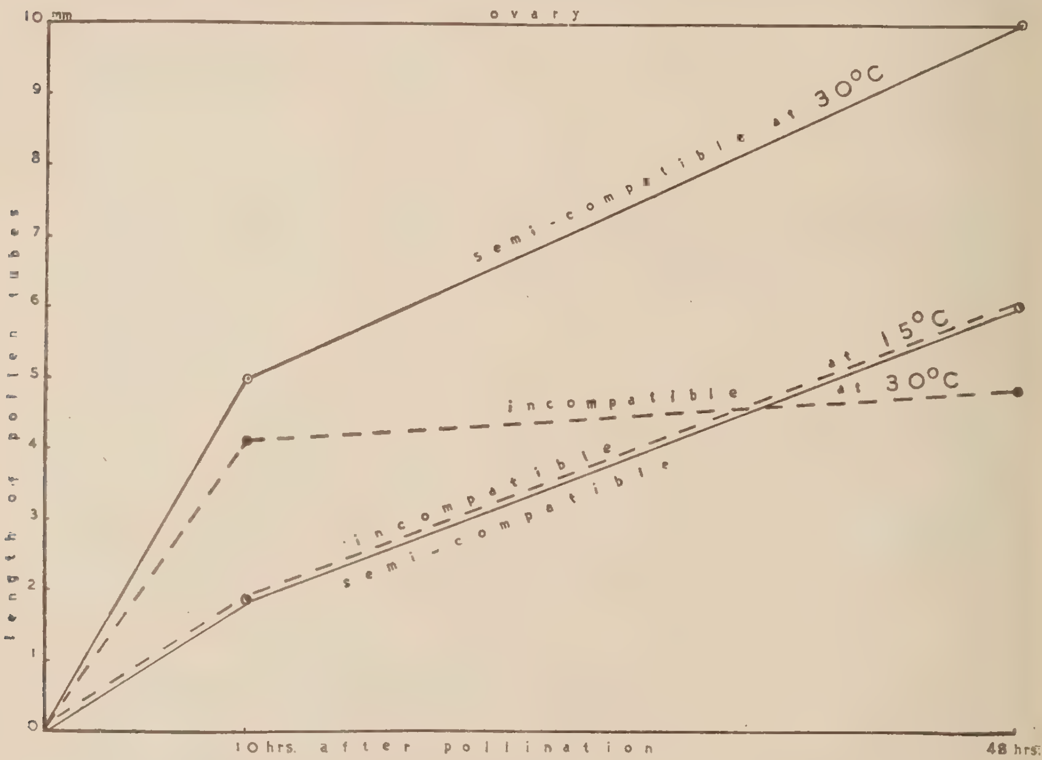

FIG. 1.
Pollen tube growth of Baumann's Reinette at 15° C. and 30° C. in 10 and 48 hours after pollination.

ends and had ceased growth as shown in Plate I, Fig. 1, and in Table III. This is a common feature of incompatible tubes in many plants.

The growth rate of the semi-compatible tubes in Cox's Orange was always higher than in Baumann's Reinette (Fig. 2 and Table II). Indeed, the high rate of pollen tube growth of Cox's Orange in its own style was exceptional. In the five other self-incompatible varieties examined, the rate was much slower. In spite of this rapidity of growth of Cox's Orange pollen tubes, no formation of proembryos, endosperm or even fertilization was observed on the sixth and thirteenth day after pollination. During this period the ovaries of Cox's Orange had ceased growing and the fruits had fallen from the tree, while those of Baumann's Reinette were continuing to develop.

In other varieties (e.g. Northern Spy and N.789) when self-pollinated, only the first type of tube was present. This was probably also the case in Böhmischer Rosenapfel (Osterwalder,

1910) and in Early McIntosh (Weeks and Latimer, 1939), in which the growth of all pollen tubes was arrested at a point about one-third of the way down the style.

It is evident from a comparison of these different varieties that there is a relationship between the degree of incompatibility and the occurrence of semi-compatible tubes, but other factors such as parthenocarpy, zygotic lethals and environment are also involved. In Cox's Orange it is possible that a greater stimulation resulting from pollination and fertilization is required to prevent fruit abscission.

The effect of temperature on the two types of tubes in Cox's Orange and Baumann's Reinette is shown in Figs. 1 and 2. During the first ten hours after germination the growth rate of both

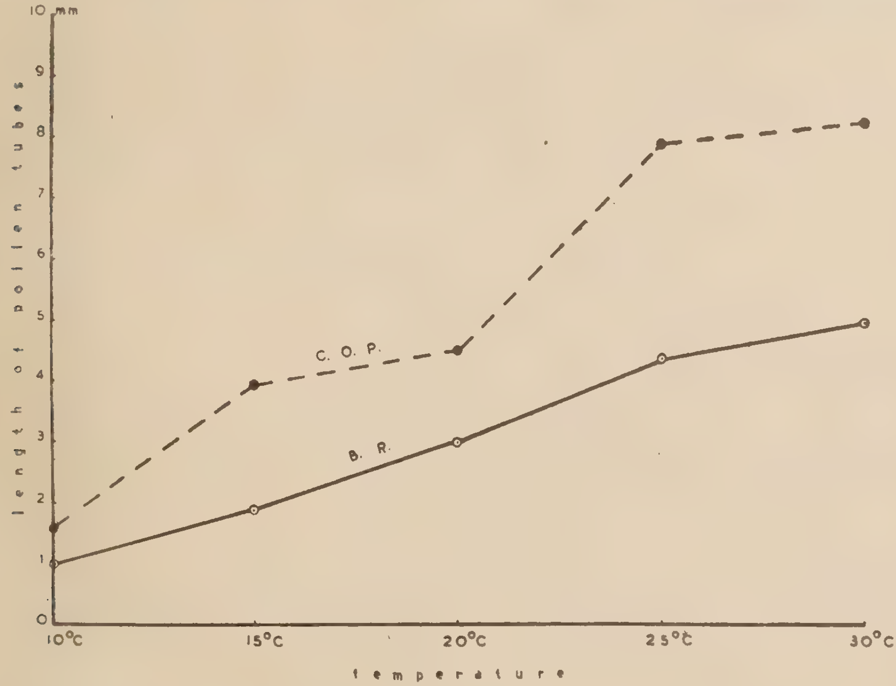

FIG. 2.

Pollen tube growth at different temperatures 10 hours after pollination.
———— Baumann's Reinette self-pollinated.
– – – – Cox's Orange self-pollinated.

kinds of tubes increases with a rise in temperature. But after ten hours the growth of incompatible tubes ceases at 25° C.-30° C., while at a lower temperature growth continues at a slow pace. This is a typical effect of temperature in incompatible pollen tube growth.

(*b*) *Self-incompatibility in triploids.*—Self-incompatibility is less frequent and not so strongly expressed in triploid apples as in diploids. For this reason, in spite of considerable gametic sterility, selfed triploids usually set a higher percentage of fruit than selfed diploids.

Blenheim Orange was investigated as a representative of self-incompatible, and Bramley's Seedling as one of relatively self-compatible triploid varieties (see Table I).

As a result of meiotic irregularities triploids have a lower percentage of " good " pollen than diploids, and this holds true also for Blenheim Orange, 69 per cent. (57-65 per cent., Wanscher, 1939) and Bramley's Seedling, 59 per cent. (52-54 per cent., Wanscher, 1939). According to Florin (1927) the pollen germination of triploids does not exceed 30 per cent.

Ten hours after pollination the pollen tubes of Blenheim Orange were shorter than those of Bramley's Seedling at all temperatures (Table II). Forty-eight hours after pollination a unimodal distribution of the tubes was found in Blenheim Orange, and even at the most favourable temperatures only three tubes in nine styles were found entering the ovary. These few tubes are presumably responsible for the 1·3 per cent. of fruit set in this variety (Table I). In Bramley's Seedling bimodality was clearly shown after forty-eight hours, indicating the presence of both incompatible and compatible tubes. The compatible tubes were found in the ovary at temperatures of 20° C.-30° C. and their compatible nature was confirmed by the 4·8 per cent. fruit set (Table I). In both Blenheim Orange and Bramley's Seedling, a percentage of tubes with swollen ends varying from 0·0 to 27·7 was found. This is considerably lower than in the diploids previously described (Table III).

TABLE III.

Number of pollen tubes per style and per cent. of swollen-ended tubes forty-eight hours after pollination.

Variety.	Temperature.									
	10° C.		15° C.		20° C.		25° C.		30° C.	
	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.
Diploids :										
Cox's Orange Pippin ..	42·7	6·2	21·3	16·9	27·3	15·2	34·7	42·3	25·3	52·6
Northern Spy	22·5	30·9	23·7	21·1	19·0	59·6	50·7	55·3	—	—
Lane's Prince Albert ..	12·2	20·4	42·7	56·2	19·0	46·1	21·0	57·2	11·3	23·5
N.789	27·5	32·7	22·7	39·7	77·7	64·3	59·0	66·7	71·3	60·7
Early Victoria ..	10·3	0·0	—	—	28·0	67·9	15·7	34·8	21·0	18·3
Baumann's Reinette ..	27·7	20·5	30·3	39·6	35·0	43·8	38·3	34·8	5·7	41·2
Mean	20·0	20·9	29·8	39·1	35·7	56·3	36·9	49·8	27·3	35·9
Triploids :										
Blenheim Orange ..	—	—	10·3	9·7	5·7	0·0	6·7	20·0	4·7	7·1
Bramley's Seedling ..	11·7	11·4	18·0	17·0	15·7	27·7	8·5	14·7	9·7	25·6
Mean	11·7	11·4	14·1	13·3	10·7	13·8	7·6	17·3	7·2	16·3

Six days after selfing, zygotes and several endosperm nuclei were formed in Bramley's Seedling embryo-sacs, but no pollen tubes or embryonic development were found in those of Blenheim (Plate I, Figs. 2 and 3).

Clearly the degree of self-compatibility in these triploid varieties is related to pollen tube growth.

(*c*) *Incompatibility between varieties.*—Complete cross-incompatibility in apples is rare, but the number of known cases of cross-incompatibility between varieties has increased considerably in recent years. This is due partly to bud mutations which have given rise to new varieties (sub-clones). All the diploid bud mutations so far tested are incompatible with the original variety, thus showing that the mutations have not affected the incompatibility genes. Furthermore, the breeding of new varieties has largely been confined to a few parental types, thus giving rise to a number of closely related seedlings. Cross-incompatibility is expected to be frequent among them and also in the back-crosses of these seedlings with their parents. This has been found to be the case as shown in Table IIIA.

The occurrence of both incompatible and compatible seedlings, when back-crossed with the male parent, is to be expected on the genetical basis found in other plants. However,

the incompatibility of seedlings when used as pollinators on the female parent is not to be expected, since the incompatibility gene, which came from the male parent and was compatible on the female parent, should segregate in the pollen of all the seedlings. This anomalous behaviour has previously been reported only in *Verbascum phoeniceum* (Sirks, 1926).

TABLE IIIA.

Segregation of incompatibility in F_1 *apple seedlings when inter-crossed and back-crossed with their parents.* (Data from the J.I.H.I.)

Parentage.		Compatible.	Incompatible.
Margil × Cox's Orange Pippin	back-cross to the male parent	10	9
Northern Spy × M. II		1	0
		11	9
Cox's Orange Pippin × Sturmer Pippin	back-cross to the female parent	1	2
Lord Grosvenor × M. Niedzwetzkyana		3	0
Northern Spy × M. II		4	2
		8	4
Lord Grosvenor × M. Niedzwetzkyana	sib-crosses	2	0
Northern Spy × M. II		10	1
		12	1

The cases of cross-incompatibility between varieties recorded in the literature are collected under different headings in Table IV. One important feature of the data is that no example of " one-way " cross-incompatibility has been found between diploid varieties. Thus reciprocal crosses behave essentially in the same way, although slight differences may exist as will be shown later. Unfortunately, it is not always clear from the literature whether the reciprocal cross had been made, but in all cases where this had been done the two crosses were alike.

In the examples of cross-incompatibility between triploids and diploids shown in Table IV, it is difficult to say whether the incompatibility is " one-way " or " reciprocal ", because of the high degree of pollen sterility in triploids. However, in the crosses involving the varieties Arkansas and Paragon there is definite evidence that the incompatibility is " one way " (Einset, 1930). This is to be expected, since the triploid parent may contain an (S) gene not present in the diploid ; and it has also been found in pears. For example, Beurré d'Amanlis (3x) × Conference (2x) fails, but in the reciprocal cross it is effective.

For a comparative study of self- and cross-incompatibility in apples two diploid varieties were chosen : Northern Spy and its seedling N.789 (Northern Spy × M. II). They had already been investigated by Crane *et al.* (1936); whose data are quoted in Table V. For control pollination Cox's Orange was used, as being compatible with both Northern Spy and N.789.

The results of pollen tube growth measurements at ten hours in self- and cross-incompatible pollinations and in a compatible control pollination are given in Fig. 3 and Table VI. It is evident that the pollen tube growth is similar in the self- and cross-incompatible pollinations. This similarity is maintained at forty-eight hours (Fig. 4). At this time in both N.789 selfed and N.789 × Northern Spy no pollen tubes have reached the ovary. However, in the reciprocal cross, many semi-compatible tubes have entered the ovary and some have penetrated the embryo-sacs. This difference is reflected in the seed set from these crosses. The number of fruits and seeds developed in both crosses is very low, but it is higher in Northern Spy × N.789 (Table V). The entry of pollen tubes into the embryo-sac in the cross Northern Spy × N.789 is not an exception, since the same behaviour was found in selfed Cox's Orange.

Table IV.

Incompatible pollinations in apples.

		Bud mutations.	
	Delicious	△Richared	Overholzer and Overley, (1932)
	,,	△Shotwell	,, ,, ,,
	,,	△Starking	,, ,, ,,
	Duchess	□Daniels' Red Duchess	Gourley and Howlett, (1941)
2x	,,	□Van Bureau Red Duchess	,, ,, ,,
	Jonathan	□Jonared	,, ,, ,,
	,,	□Blackjon	,, ,, ,,
	McIntosh	□Blackmack	,, ,, ,,
	Northern Spy	□Red Spy	,, ,, ,,
	Rome Beauty	□Stark's Dark Red Rome	Howlett, (1933)
	Stayman Winesap (3x)	□Blaxtayman (3x)	Gourley and Howlett, (1941)
		Seedlings.	
			McDaniels and Burrel, (1934)
	Cortland	△Early McIntosh	Latimer, (1937)
	(B. Davis × McIntosh)	(Yellow Transparent × McIntosh)	Weeks and Latimer, (1939)
2x	Northern Spy	△N.789 (Northern Spy × M. II)	Crane *et al.*, (1936)
	Rome Beauty	△Galia Rome Beauty	Howlett, (1933)
	,, ,,	△N.73	,, ,,
	Ribston Pippin (3x)	⊗Cox Pomona	Johansson, (1926)
		Varieties of unknown relation.	
	Berner Rosenapfel	△Parkers Peping	Kobel *et al.*, (1939)
	Chusenrainer	△Transparent v. Croncels	,, ,,
2x	Goldparmäne	△Sauergrauech	,, ,,
	Oberrieder Glanzrenette	△Oetwiler Renette	,, ,,
	Red Astrachan	□Oranie	Callmar and Johansson, (1935)
	Yellow Richard	□Filippa	,, ,, ,,
	Arkansas	⊙Grimes Golden	Auchter and Schrader, (1926)
	Paragon	⊙ ,, ,,	Knowlton, (1927)
	Blenheimer Goldrenette	⊗Goldparmäne	Rudloff and Schanderl, (1937)
	,, ,,	⊗Sauergrauech	Kobel *et al.*, (1939)
	Jakob Lebel	⊗Goldparmäne	,, ,,
	,, ,,	⊗Sauergrauech	,, ,,
3x	Kanada Renette	⊗Goldparmäne	Rudloff and Schanderl, (1937)
	,, ,,	⊗Weisser Winter Calville	,, ,, ,,
	,, ,,	⊗Berner Rosenapfel	Kobel *et al.*, (1939)
	,, ,,	⊗Sauergrauech	,, ,,
	,, ,,	⊗Transparent v. Croncels	,, ,,
	Schöner v. Boskoop	⊗Chusenrainer	,, ,,
	,, ,,	⊗Transparent v. Croncels	Rudloff and Schanderl, (1937)

△ Both ways incompatible.
⊙ One way incompatible.
⊗ Probably one way incompatible but results obscured by pollen sterility.
□ No record of reciprocal cross.

Table V.

Setting of fruit and seed in Northern Spy and N.789. (Crane *et al.*, 1936 ; *Brittain, 1933.)

Varieties.	No. of flowers.	Fruits.		Seeds.	
		No.	%	No. per fruit.	%
Northern Spy selfed	3,385	24	0·7	6	0·25
N.789 selfed	104	2	1·9	2	1·00
N.789 × Northern Spy	420	5	1·2	6	1·20
Northern Spy × N.789	121	2	1·6	5	2·50
Northern Spy × Cox's Orange* ..	5,843	842	14·58	—	—

The effect of temperature on pollen tube growth at ten hours is shown in Fig. 3 and at forty-eight hours in Fig. 4, from which it is evident that inhibition of pollen tube growth by incompatibility is greater at high than at low temperatures. For example, in the styles of N.789 both its own pollen tubes and those of Northern Spy are completely inhibited at 25° C. about ten hours after pollination, while at a lower temperature growth still continues after this time. In the styles of Northern Spy inhibition of its own and of N.789 pollen tubes occurs a little later, as some growth is recorded after ten hours.

Six days after pollination the ovaries of the flowers pollinated with compatible pollen of Cox's Orange were developing, and most of the embryo-sacs had been penetrated by pollen

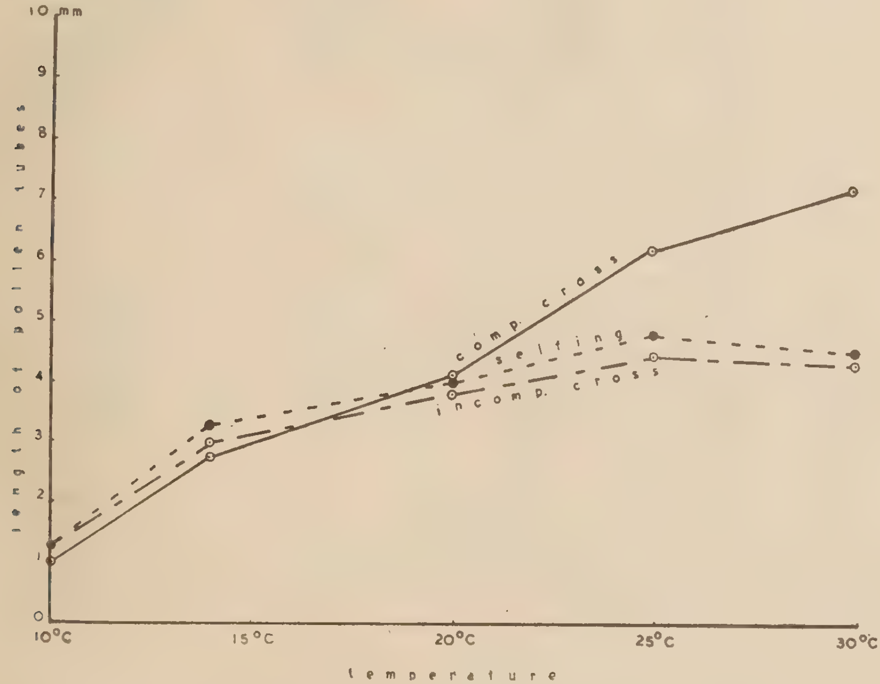

FIG. 3.

Pollen tube growth at different temperatures 10 hours after pollination.
- - - - N.789 self-pollinated.
— — — N.789 × Northern Spy—incompatible.
——— N.789 × Cox's Orange—compatible.

tubes. In selfed and incompatibly crossed flowers at this time many of the ovules had begun to shrink. In some of the embryo-sacs, though normal in appearance, the synergides were often degenerating. No tubes were found, except one entering the ovary in N.789 × Northern Spy.

Twelve days after pollination the fruitlets from crossing with Cox's Orange pollen were rapidly growing, and the 4- to 8-celled proembryos were surrounded with polynucleate endosperm. Most of the selfed and incompatibly crossed flowers had abscissed, and those left had shrunken ovules and embryo-sacs.

From the behaviour of pollen tube growth for different periods of time and at different temperatures, and also from the embryological observations, it is evident that self- and cross-incompatibility are essentially of the same nature. They depend on the same kind of reaction

caused by the same incompatibility genes. The different degrees of incompatibility occurring both on selfing and crossing are probably due to different combinations of (S) genes and to combinations of polygenes which weaken the reaction of some pollen tubes, called here semi-compatible.

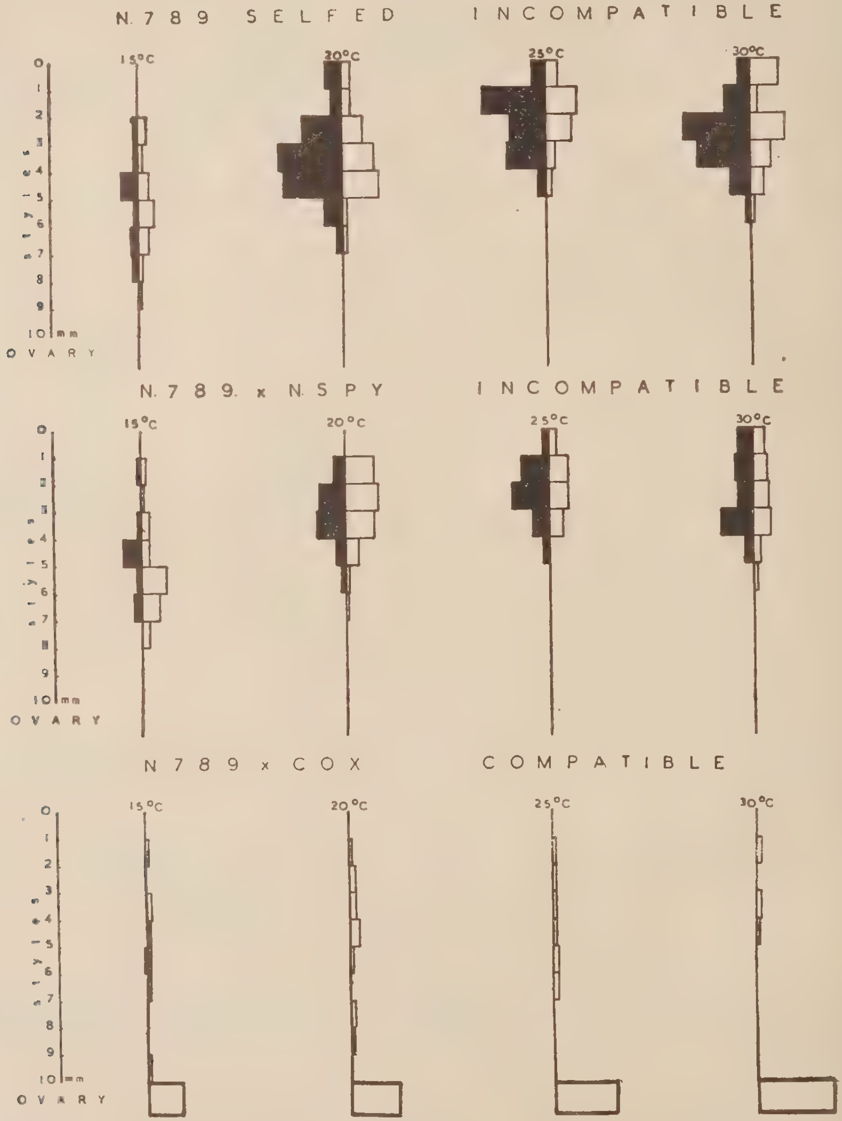

FIG. 4.

Distribution of pollen tubes in the styles of N.789, 48 hours after pollination.

normal tubes.

swollen tubes.

The number of tubes in the ovary of the compatible cross is an approximation.

TABLE VI.

Pollen tube growth ten hours after pollination.

Variety.	Temperature. 10° C.	15° C.	20° C.	25° C.	30° C.
	mm.	mm.	mm.	mm.	mm.
Selfing :					
N.789	1·3 (8)	3·2 (6)	3·9 (8)	4·6 (10)	4·3 (11)
Northern Spy	1·2 (5)	2·5 (4)	3·1 (5)	4·0 (10)	5·0 (9)
Crossing :					
Incompatible :					
N.789 × Northern Spy	1·3 (6)	2·9 (8)	3·7 (7)	4·2 (8)	3·7 (11)
Northern Spy × N.789	0·8 (5)	2·7 (6)	3·0 (6)	4·2 (9)	4·1 (10)
Compatible :					
Cox's Orange Pippin × N.789	— —	2·7 (3)	4·5 (3)	6·9 (3)	6·9 (3)
Cox's Orange Pippin × Northern Spy ..	— —	2·8 (3)	4·8 (3)	7·5 (3)	6·3 (3)
N.789 × Cox's Orange Pippin	1·0 (8)	2·7 (8)	4·0 (7)	6·0 (10)	6·9 (8)
Northern Spy × Cox's Orange Pippin ..	1·0 (6)	2·8 (12)	3·6 (9)	6·2 (8)	6·9 (8)

(Figures in brackets indicate the number of styles examined.)

CROSS-COMPATIBILITY IN APPLES.

Most cross-pollinations between varieties of apples are compatible but not all are productive, owing to gametic sterility. In diploid apples sterility is negligible while in triploids it is high, owing to abnormal chromosome balance in their gametes. Four groups of compatible cross-pollinations were investigated: (1) diploids × diploids, (2) triploids × diploids, (3) diploids × triploids, (4) triploids × triploids.

TABLE VII.

Number of pollen tubes per style and per cent. of swollen-ended tubes forty-eight hours after pollination.

Variety.	Temperature. 10° C.		15° C.		20° C.		25° C.		30° C.	
	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.
Selfing :										
N.789	27·5	32·7	22·7	39·7	77·7	64·3	59·0	66·7	71·3	60·7
Northern Spy	22·5	30·9	23·7	21·1	19·0	59·6	50·7	55·3	—	—
Crossing :										
Incompatible :										
N.789 × Northern Spy	25·3	48·7	27·0	28·4	45·0	39·3	37·3	59·8	37·3	55·4
Northern Spy × N.789	—	—	31·7	35·8	58·7	58·0	25·5	66·7	—	—
Compatible :										
Cox's Orange Pippin × N.789 ..	—	—	28·3	10·5	18·7	14·3	7·3	4·5	0·0	0·0
Cox's Orange Pippin × Northern Spy	—	—	7·3	0·0	8·0	20·8	5·0	26·7	1·0	75·0
N.789 × Cox's Orange Pippin ..	—	—	4·3	7·7	6·3	0·0	2·7	0·0	1·7	0·0
Northern Spy × Cox's Orange Pippin	—	—	9·7	6·9	1·7	0·0	8·7	50·0	0·0	0·0

(1) *Diploids × diploids.*—In this group the pollen of Cox's Orange was used on five other diploid varieties; the five reciprocal pollinations were also made. It was found that the pollen tube growth of Cox's Orange was similar in the styles of the five varieties used, and it increased more or less regularly with a rise in temperature (Table VIII).

In the reciprocal pollinations, i.e. Cox's Orange styles with pollen of the five other varieties, there was some variation in the rate of pollen tube growth, especially at the higher temperatures (25° C. and 30° C.). The pollen tubes of Early Victoria grew the most rapidly, while those of Baumann's Reinette were the slowest. The mean pollen tube growth of these five varieties was constantly higher at all temperatures than that of Cox's Orange in reciprocal crosses, but

TABLE VIII.

Pollen tube growth in compatible crosses ten hours after pollination.

Varieties.	Temperature.				
	10° C. mm.	15° C. mm.	20° C. mm.	25° C. mm.	30° C. mm.
Diploid × diploid :					
Baumann's Reinette × Cox's Orange Pippin	1·2 (3)	1·8 (5)	3·2 (5)	6·2 (6)	5·4 (8)
Lane's Prince Albert × Cox's Orange Pippin	1·2 (4)	2·6 (8)	2·9 (9)	6·6 (7)	6·8 (4)
Early Victoria × Cox's Orange Pippin ..	1·3 (4)	3·0 (3)	— —	6·3 (3)	6·8 (3)
N.789 × Cox's Orange Pippin	1·0 (8)	2·7 (8)	4·0 (7)	6·0 (10)	6·9 (8)
Northern Spy × Cox's Orange Pippin ..	1·0 (6)	2·8 (12)	3·6 (9)	6·2 (8)	6·9 (8)
Mean (Cox's Orange Pippin ♂)	**1·14**	**2·58**	**3·42**	**6·26**	**6·56**
Cox's Orange Pippin × Baumann's Reinette	1·0 (8)	3·1 (4)	4·1 (8)	5·3 (8)	6·4 (6)
Cox's Orange Pippin × Lane's Prince Albert	1·6 (8)	3·0 (8)	4·6 (5)	6·7 (7)	7·6 (5)
Cox's Orange Pippin × Early Victoria ..	1·6 (7)	3·3 (7)	4·9 (7)	7·5 (7)	8·2 (8)
Cox's Orange Pippin × N.789	— —	2·7 (3)	4·5 (3)	6·9 (3)	6·9 (3)
Cox's Orange Pippin × Northern Spy ..	— —	2·8 (3)	4·8 (3)	7·5 (3)	6·3 (3)
Mean (Cox's Orange Pippin ♀)	**1·4**	**2·98**	**4·58**	**6·78**	**7·08**
Total mean (Cox's Orange Pippin ♂ and ♀)	**1·27**	**2·78**	**4·00**	**6·52**	**6·82**
Triploid × diploid :					
Blenheim Orange × Cox's Orange Pippin ..	0·5 (5)	2·1 (3)	3·8 (3)	6·3 (4)	6·9 (3)
Bramley's Seedling × Cox's Orange Pippin	1·4 (3)	2·7 (3)	4·2 (3)	7·1 (3)	7·5 (5)
Mean	**0·95**	**2·4**	**4·0**	**6·7**	**7·2**
Diploid × triploid :					
Cox's Orange Pippin × Blenheim Orange ..	0·7 (7)	1·5 (8)	2·8 (10)	4·2 (11)	4·7 (8)
Cox's Orange Pippin × Bramley's Seedling	0·8 (8)	1·3 (11)	2·1 (10)	4·3 (10)	4·3 (9)
Mean	**0·75**	**1·4**	**2·45**	**4·25**	**4·5**
Triploid × triploid :					
Blenheim Orange × Bramley's Seedling ..	0·3 (5)	1·3 (7)	2·0 (10)	2·9 (8)	3·9 (8)
Bramley's Seedling × Blenheim Orange ..	0·9 (4)	1·9 (7)	2·3 (6)	3·1 (7)	5·4 (7)
Mean	**0·6**	**1·6**	**2·15**	**3·0**	**4·65**

(Figures in brackets indicate the number of styles examined.)

the difference, although not very considerable, was significant at 15° C. ($P = 0{\cdot}02 - 0{\cdot}01$) and at 30° C. ($P < 0{\cdot}01$).

In the compatible pollinations some had no (or very few) swollen tubes ten hours after pollination. After forty-eight hours all had some swollen-ended tubes, but the proportion differed in different pollinations. There was also great variation in the proportion of tubes which developed swollen ends at different temperatures. The number of swollen tubes was always much lower in compatible cross-pollinations than in self-pollinations.

Some of the pollen tubes present in the styles forty-eight hours after pollination may still have a chance of reaching the ovary at temperatures between 15° C. and 25° C. At 30° C., however, a bimodality of pollen tube growth is evident ; the growth of some tubes is checked, and these have no chance of growing the full length of style. The clear-cut difference between the growth of these and that of compatible tubes indicates that the growth of the former is arrested. This cessation, accompanied by swelling, suggests that it is caused by a genic incompatibility reaction ; but the possibility of starvation owing to the competition of a large number of pollen tubes, as in *Primula obconica* (Modlibowska, 1942), cannot entirely be ruled out.

In compatible crosses between diploids a proportion of the pollen tubes reached the ovaries forty-eight hours after pollination at all temperatures between 15° C. and 30° C. At 25° C. pollen tubes penetrated some embryo-sacs, and even two endosperm nuclei were found. At 30° C. the endosperm was further developed, and up to 16 nuclei were counted.

Six days after pollination different stages of embryonic development were seen. In some of them fertilization of the egg and triple fusion had occurred, in others a dividing zygote was visible, in others again 2-celled proembryos were present.

TABLE IX.

Number of pollen tubes per style and per cent. of swollen-ended tubes forty-eight hours after pollination.

Varieties crossed.	Temperature.									
	10° C.		15° C.		20° C.		25° C.		30° C.	
	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.
Diploid × diploid :										
Baumann's Reinette × Cox's Orange	11·0	0·0	0·0	0·0	1·7	0·0	9·0	22·2	27·3	40·2
Lane's Prince Albert × Cox's Orange	9·7	0·0	5·3	6·2	2·7	0·0	2·7	25·0	6·3	15·8
Early Victoria × Cox's Orange ..	12·3	0·0	—	—	6·3	5·3	2·3	0·0	3·3	0·0
N.789 × Cox's Orange	—	—	4·3	7·7	6·3	0·0	2·7	0·0	1·7	0·0
Northern Spy × Cox's Orange ..	—	—	9·7	6·9	1·7	0·0	8·7	50·0	—	—
Mean (Cox's Orange ♂)	**11·0**	**0·0**	**4·8**	**5·2**	**3·7**	**1·1**	**5·1**	**19·4**	**9·4**	**14·0**
Cox's Orange × Baumann's Reinette	7·7	0·0	11·0	3·0	4·0	0·0	9·0	29·6	7·7	34·8
Cox's Orange × Lane's Prince Albert	29·3	0·0	3·0	0·0	14·3	2·3	4·0	0·0	1·7	0·0
Cox's Orange × Early Victoria ..	16·4	0·0	4·7	0·0	2·7	0·0	0·3	0·0	6·7	25·0
Cox's Orange × N.789	—	—	28·3	10·5	18·7	14·3	7·3	4·5	0·0	0·0
Cox's Orange × Northern Spy ..	—	—	7·3	0·0	8·0	20·8	5·0	26·7	1·0	75·0
Mean (Cox's Orange ♀)	**17·1**	**0·0**	**10·9**	**2·7**	**9·5**	**7·5**	**5·1**	**12·2**	**3·4**	**27·0**
Total mean (Cox's Orange ♂ and ♀)	**14·0**	**0·0**	**7·8**	**3·9**	**6·6**	**4·3**	**5·1**	**15·8**	**6·4**	**20·5**
Triploid × diploid :										
Blenheim Orange × Cox's Orange ..	—	—	10·3	12·9	0·0	0·0	5·0	6·7	1·0	0·0
Bramley's Seedling × Cox's Orange	6·0	0·0	7·7	8·7	3·4	0·0	18·3	16·4	4·3	15·4
Mean	**6·0**	**0·0**	**9·0**	**10·8**	**1·7**	**0·0**	**11·6**	**11·5**	**2·6**	**7·7**
Diploid × triploid :										
Cox's Orange × Blenheim Orange ..	3·8	5·3	9·7	0·0	5·0	20·0	3·0	11·1	0·3	0·0
Cox's Orange × Bramley's Seedling	9·7	10·3	6·7	20·0	9·0	11·1	3·0	22·2	1·2	20·0
Mean	**6·7**	**7·8**	**8·2**	**10·0**	**7·0**	**15·5**	**3·0**	**16·6**	**0·7**	**10·0**
Triploid × triploid :										
Blenheim Orange × Bramley's Seedl.	—	—	13·0	17·2	3·7	9·1	4·7	21·4	8·3	52·0
Bramley's Seedl. × Blenheim Orange	8·5	8·8	10·0	22·0	20·7	24·2	8·2	36·4	6·4	25·0
Mean	**8·5**	**8·8**	**11·5**	**19·6**	**12·2**	**16·6**	**6·4**	**28·9**	**7·3**	**38·5**

Twelve days after pollination 4- to 12-celled proembryos were found in many embryo-sacs. The endosperm consisted of about 30-60 nuclei, dispersed along the walls of the embryo-sac and connected with plasmatic strands. The endosperm nuclei were grouped more densely around the proembryo at the micropylar and the chalazal ends than along the middle part of the embryo-sac wall.

(2) *Triploids × diploids.*—Two triploid varieties—Blenheim Orange and Bramley's Seedling—were pollinated with Cox's Orange Pippin. The pollen germinated well on the stigmas of both varieties. The rate of pollen tube growth of Cox's Orange was consistently lower in the styles of Blenheim Orange at all temperatures than in the styles of Bramley's Seedling (see Table VIII). In both styles, however, growth was accelerated with a rise in temperature.

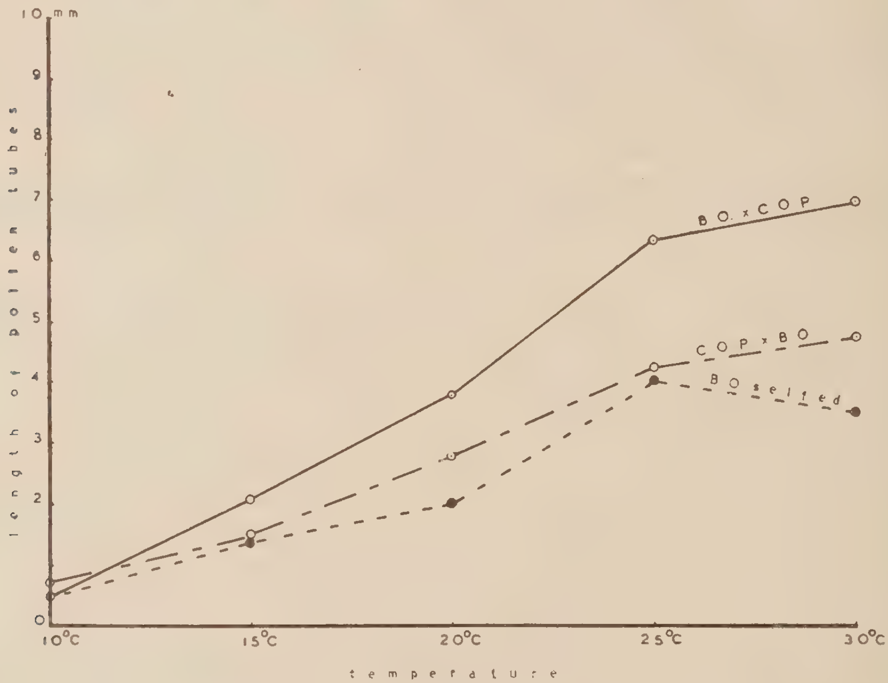

FIG. 5.

Pollen tube growth 10 hours after pollination.

———— Blenheim Orange × Cox's Orange.

— — — — Cox's Orange × Blenheim Orange.

- - - - - Blenheim Orange selfed.

Swelling of some pollen tubes occurred ten hours after pollination at 25° C. and 30° C. Forty-eight hours after pollination the percentage of swollen tubes rose, but on the whole it was lower in these triploid × diploid pollinations than in triploid self-pollinations (Tables IX and III).

At that time pollen tubes had entered the ovaries at all temperatures ranging from 15° C. to 30° C. At 20° C. they were seen in the locules of Bramley's Seedling; at 25° C. in the embryo-sacs; at 30° C. the first division of the zygote and 4-6 nuclei of the endosperm were present.

Six days after pollination, up to 16 endosperm nuclei were seen in the embryo-sacs of Blenheim Orange, and twelve days after pollination proembryos were found in both Blenheim Orange and Bramley's Seedling. In the latter mostly 8- to 10-celled proembryos were seen (Plate II, Fig. 4).

Thus in all main respects pollen tube growth and embryo development was the same as in diploid×diploid compatible pollinations.

(3) *Diploids×triploids.*—In this group the same varieties were studied as in the triploid× diploid group, but in reciprocal combinations. In the styles of Cox's Orange the pollen tube growth of both Blenheim Orange and Bramley's Seedling was very similar (Table VIII). In both, however, it was much slower (particularly at high temperatures) than in any diploid× diploid and triploid×diploid combination of varieties. The mean growth of Blenheim Orange

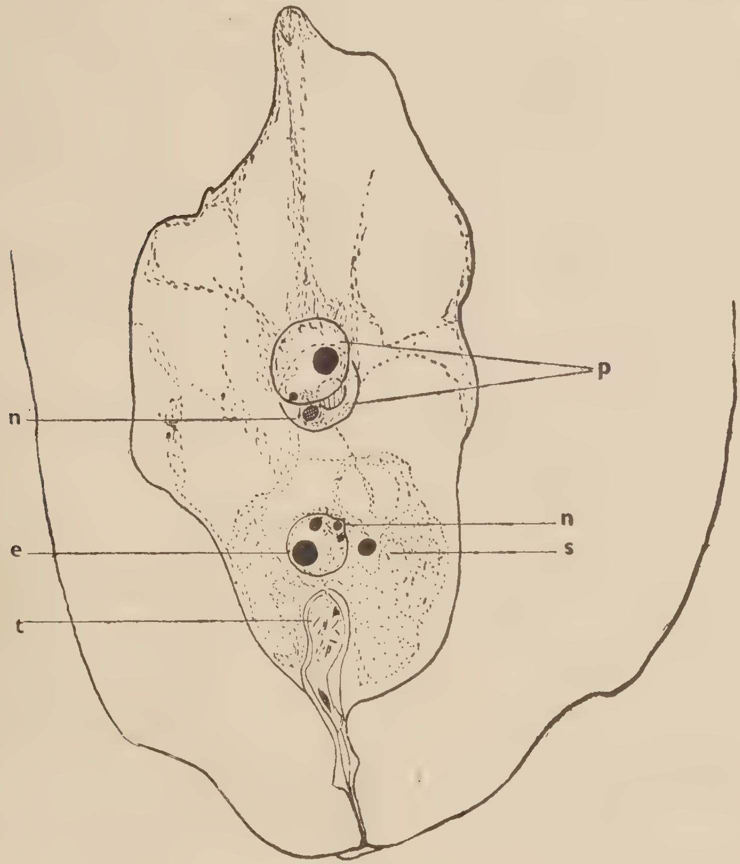

FIG. 6.

Embryo-sac of Bramley's Seedling ×Blenheim Orange 6 days after pollination. t—pollen tube, e—fertilized egg, s—synergides, n—sperm nucleus, p—polar nuclei.

and Bramley's Seedling tubes in Cox's Orange styles closely approximated that in the styles of these two varieties when self-pollinated (Fig. 5).

Forty-eight hours after pollination the pollen tubes had reached the ovary at 15° C.-30° C. None, however, had reached the ovules at this time. Many of the ovules at 25° C. had begun to shrink (Plate II, Figs. 5 and 6). The number of tubes remaining in the style was small and a fair percentage of them had swollen ends. Twelve days after pollination 3- to 8-celled pro-embryos were present in Cox's Orange, both when pollinated with Bramley's Seedling and with Blenheim Orange. In the latter several degenerated embryo-sacs were seen. On the whole

in diploids × triploids pollen tube growth and embryo development were slower than in the reciprocal crosses.

(4) *Triploids × triploids.*—Blenheim Orange and Bramley's Seedling were reciprocally inter-crossed. Bramley's Seedling pollen tubes grew consistently more slowly at all temperatures in Blenheim Orange styles than Blenheim Orange tubes did in Bramley's Seedling styles (Table VIII). The inhibition of Bramley's Seedling pollen tubes in Blenheim Orange styles resembled that of Blenheim Orange on selfing, although the pollen tubes of Bramley's Seedling in its own styles had a higher growth rate (Table II). This difference was also evident forty-eight hours after pollination, at 15° C., when the longest tubes of Blenheim Orange had reached the ovaries of Bramley's Seedling, while in the reciprocal cross the tubes did not traverse the full length of styles. When pollen of these two varieties was used on Cox's Orange styles, the pollen tube growth was approximately the same. Thus, in the reciprocal cross, Blenheim Orange styles had some inhibitory influence on Cox's Orange pollen tubes. Clearly the Blenheim Orange style is an unfavourable medium for its own pollen and for that of diploids and triploids.

Six days after pollination most of the Blenheim Orange flowers pollinated by Bramley's Seedling had dropped from the trees. No pollen tubes and only shrunken ovules were found in the ovaries of those still remaining on the trees. In the reciprocal cross some fruitlets had developed considerably. Pollen tubes were found in the ovaries, and in some of the embryo-sacs the zygote and the endosperm nuclei were present (Fig. 6).

Comparing the four different compatible crosses studied, it is evident that the rate of pollen tube growth is very largely determined by the chromosome number of their pollen parent. The pollen tubes of the diploid varieties grow more quickly at all temperatures and are much more accelerated by increased temperature than those of the triploid varieties (Fig. 7).

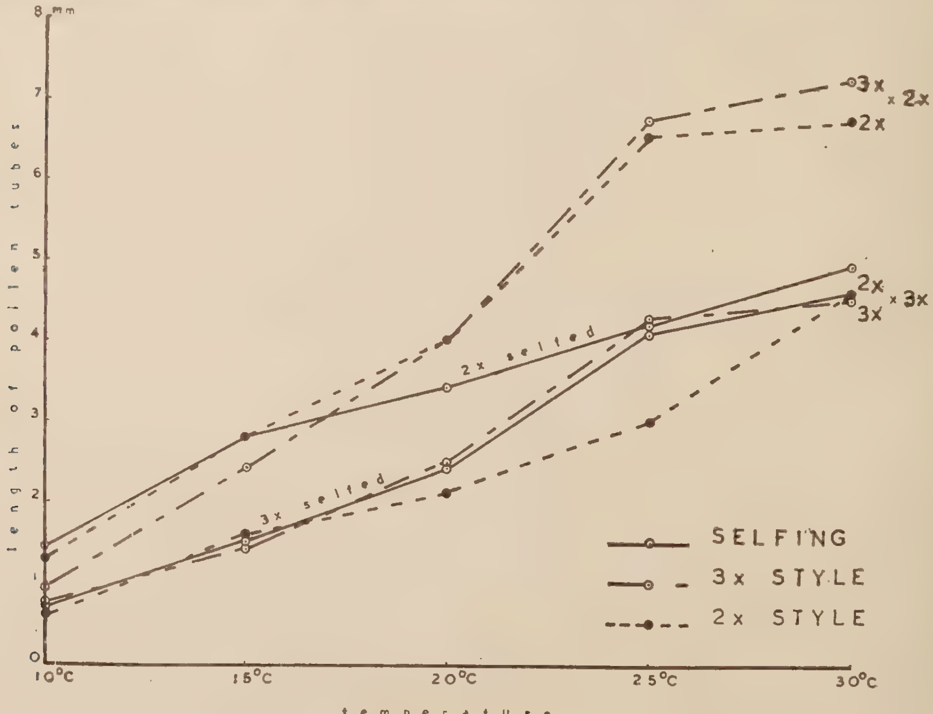

FIG. 7.
Pollen tube growth of diploid and triploid varieties 10 hours after pollination.

These two types of pollen tubes show this difference in growth in the styles of both diploid and triploid varieties. This is in agreement with the results obtained by Buchholz and Blakeslee (1929 and 1941) in Datura *sp.* Thus, they found that the pollen of diploids usually grew more rapidly in the styles of diploids, triploids and tetraploids than did the pollen of tetraploids. In tomatoes, also, I have found that the pollen of diploids grows more rapidly than that of tetraploids, both in diploid and tetraploid styles (Modlibowska, unpublished).

The percentage of fruit set in apples follows a similar trend. According to Brittain (1933), and Brittain and Eidt (1933), a higher fruit set is obtained from diploid and triploid varieties when pollinated with the pollen of a diploid variety than when that of a triploid is used. They found no significant difference between the diploid × triploid and triploid × triploid crosses. The data presented by Crane and Lewis (1942) also show that pollination with diploid varieties induces a higher percentage of fruit than with triploids (3x × 3x compared with 3x × 2x, and 2x × 3x with 2x × 2x).

There is no doubt that the unbalanced gametes of triploids are responsible for much of the ineffectiveness of triploid pollen, but this does not explain why this pollen fails to stimulate the production of fruit in triploid and in some diploid varieties which have parthenocarpic tendencies. The clue to this behaviour may be found in the diploid pear Beurré Bedford. It has abnormal pollen, owing to the lack of wall formation in the pollen mother cell (Thomas, 1942). The tubes of this pollen grow rapidly down the style and enter the embryo-sac, but only a few seeds are produced. Nevertheless a high percentage of seedless fruits is formed. Thus, although the pollen contains unbalanced gametes, it has a stimulating effect on fruit formation. The lack of stimulation of fruit formation by the pollen of triploid apples appears to be due to the slowness of pollen tube growth, which, in turn, may be due to the thickness of the tubes themselves. According to Kostoff (1943, p. 711), the thickness of the pollen tubes depends on their chromosome number ; thicker pollen tubes grow more slowly through styles with a smaller chromosome number than thinner ones do. Kostoff supposes this to be a mechanical inhibition accompanied by unknown physicochemical processes.

However, within genera of plants, a relationship exists between the volume of the pollen grain and that of its tube one hour after pollination under optimum conditions (Lewis, unpublished). It is possible that in triploid apples the width of the pollen tubes increases disproportionately and thus reduces their rate of growth. The data available, however, are too few to enable a definite conclusion on this point to be reached.

In self-pollinations, a comparison of pollen tube growth of diploids and triploids confirms the slow growth of the pollen of the triploid varieties. The rate of growth of these two types differs at 15° C. because the temperature is too low for the incompatibility reaction to have effect. The rate of growth of pollen tubes from diploid varieties is then higher than that of triploid varieties. With a rise in temperature the intensity of the incompatibility reaction increases and inhibits the pollen tube growth in selfed diploids. In selfed triploids the leading tubes are compatible, no inhibition of them being observed, though they have the slow growth characteristic of pollen tubes of triploid varieties. In this way the two types of pollen tubes in their own styles attain a similar growth rate at 25° C. and 30° C.

Comparing the pollen tube growth of selfed diploid varieties with that of compatible crosses between diploids (Fig. 3 ; Table VI), it is evident that ten hours after pollination it is the same at temperatures ranging from 10° C. to 20° C., but at 25° C. to 30° C. there is a considerable difference between the two. The inhibition of pollen tube growth on selfing at the higher temperatures is due to incompatibility reactions. These reactions are almost imperceptible at lower temperatures, but they increase with a rise in temperature and thereby counteract the accelerating influence of higher temperatures. This inhibition is relatively weak, and the curve representing pollen tube growth ten hours after pollination rises steadily with the rise in temperature (Fig. 7), instead of decreasing rapidly as in other plants (*Oenothera organensis*, *Prunus avium* and *Primula obconica* (Lewis, 1942*a*)). The reason is that these plants are completely

self-incompatible, whereas in apples partial rather than complete incompatibility is the rule, and their longest tubes are often semi-compatible.

In apples the fully incompatible tubes are arrested about half way down the style at 30° C. At a temperature of 25° C., however, they travel a little farther. By this time the compatible tubes have reached the ovary and effected fertilization. The incompatible pollen tubes therefore behave in the same way as those of other self-incompatible species.

DISCUSSION.

The role of pollen tubes consists not only in carrying the sperm nuclei into the embryo-sac but also in the stimulation of ovarian growth. In most varieties fertilization is essential for the development of the fruit ; in some varieties, however, fruits can set and develop to maturity without fertilization taking place. But in both cases there seems to be a close correlation between the rate of pollen tube growth and fruit development. In all the pollinations I have investigated the pollen of diploid varieties was more effective than that of triploids. The lower efficiency of the pollen of triploid varieties is due to several causes. Firstly, a very high proportion of their pollen is sterile and fails to germinate. Secondly, many of the pollen grains which do germinate, develop tubes and effect fertilization, give rise to unbalanced zygotes in which there is a breakdown of embryonic growth at an early stage. Thirdly, the relatively slow tube growth of the pollen tubes of triploid varieties may also play a part in its inefficiency. In the pollinations I have examined, where the pollen of triploids has been used, incompatibility has played a minor part.

The part played by the pollen tubes in the stimulation of fruit development has been shown by Yasuda (1939) in many plants. He found that the stimulation can be efficient only when the pollen tubes have penetrated deeply into the styles. With regard to parthenocarpic development, on the whole, the more rapidly the pollen tubes grow down the style the earlier the stimulation of ovarian growth takes place, and the greater becomes its chance of developing into a fruit.

Bryant (1935) investigated apples, especially the diploid variety McIntosh. Using this as female, he carried out self-pollinations and made cross-pollinations with the pollen of three varieties. In all cases he found an early stimulation which retarded the breakdown of embryo-sacs and also the abscission of the flowers. The degree of stimulation and retardation, however, varied according to the pollen used. It was weakest when McIntosh was self-pollinated, and strongest when it was crossed with Delicious, a diploid variety. The pollen of Baldwin and Gravenstein, both triploid varieties, had intermediate effects.

Weeks and Latimer (1939) investigated Early McIntosh, a seedling from Yellow Transparent × McIntosh, and found that some stimulation followed self-pollination, but none when it was pollinated by the variety Cortland, a seedling from Ben Davis × McIntosh. In this case the selfed pollen tubes grew farther down the styles than did those of Cortland, a variety which is incompatible with Early McIntosh.

My measurements of the length and diameter of ovules and embryo-sacs of N.789 showed no difference, whether the styles were selfed or incompatibly cross-pollinated with Northern Spy. On the sixth day after pollination there was some initial enlargement of the ovules, although no pollen tube could be traced inside the ovary. On the twelfth day, however, all the ovules examined were shrunken and the embryo-sacs had completely degenerated. At this time a few pollen tubes were found in the ovaries, but evidently they arrived too late to exercise any stimulating or other influence.

When N.789 is compatibly pollinated with Cox's Orange, pollen tubes are found in the embryo-sacs six days after pollination. At this time the embryo-sacs and ovules of N.789 are about one and a half times larger, both in length and diameter, than when the pollinator is Northern Spy.

The failure of fruit development in the diploid varieties investigated was due to incompatibility. Thus, in N.789 × Northern Spy all the pollen tubes showed the characteristic incompatibility reaction ; their growth was arrested about half way down the style and no fruit developed. In other crosses where diploid pollen was used a proportion of the tubes effected fertilization, but others failed to travel the full length of the style and reach the ovary. That is to say, a proportion of the pollen tubes is compatible and a proportion incompatible. It is not possible at present to attempt to give a genic interpretation of the behaviour of incompatibility in apples. It is highly probable that it is controlled by genes as in other fruit trees, but the high polyploidy of apples renders both the phenotypic behaviour and the inheritance of incompatibility complex.

Incompatibility is determined by like genes failing to meet like, and the rare occurrence of cross-incompatibility in apples which are complex polyploids (partially tetraploid and partially hexaploid) is noteworthy. For it follows that the more complex the polyploidy the less frequently will complete incompatibility occur, as the chances of individuals of similar genetic constitution meeting to form incompatible groups are greatly reduced. In diploid fruits such as cherries, self-incompatibility is the rule and cross-incompatibility common. In *Prunus domestica* (hexaploid), complete self- and cross-incompatibility, though of frequent occurrence, is far less common than in cherries, and in apples it is much less frequent than in plums. This decreasing frequency of incompatibility, as chromosome number and complexity increases, is therefore in accordance with genetic expectation.

II. PEARS.

Introduction.

Pears, like apples, are considered to be secondary polyploids (Moffet, 1931 and 1934). Functionally, however, they are more like diploids than apples, and this may possibly be due to the greater differentiation of their chromosomes (Crane and Lewis, 1942). The segregation of different morphological characters is much clearer in pears than in apples (Crane and Lewis, 1940) and self-incompatibility is also more sharply defined. The diploid pear varieties are completely or almost completely self-incompatible. Out of 122 varieties examined Schanderl (1937) found only two which, on selfing, set a considerable number of fruits, but it is noteworthy that these fruits were all parthenocarpic. The tendency to parthenocarpy is widespread among pears (Reinecke, 1930). Twenty-three out of forty-one varieties described by Kobel *et al.* (1939) have this tendency in a greater or less degree. Amongst the most outstanding are : Easter Beurré (Johansson, 1931), Esperens Bergamotte (Schanderl, 1937), Neue Poiteau (Schanderl, 1937), Bartlett (2x) (Chandler, 1942), Fertility (2x) (Lewis, 1942).

For this reason the seeds set in diploid pears must always be taken into account when determining their compatibility relationships. The triploid varieties form a higher percentage of seedless fruits than the diploids ; this is largely due to unbalanced zygotes and to early breakdown of embryonic development.

Material and methods.

The material and methods used for pears were very similar to those used for apples, but with the following differences. In the experiment concerning the age of the flower, all flowers of each variety were emasculated on the same day. In these flowers the pollen tubes in all of the five styles were measured ; in others, only three styles per flower were dissected and the tubes measured. Since the pollen of pears was found to germinate freely at 10° C. no experiments were carried out at 15° C. The first measurements of pollen tube growth were made twenty-four hours after pollination.

Pollen tube growth in relation to the age of the flower.

In contrast to apples, the degree of receptivity of the styles in pears has an effect on pollen germination and tube development, and for this reason a more detailed study of this effect was made in pears. Investigations were first carried out to determine optimum time and conditions for pollination and pollen tube growth. It was found that pear flowers are dichogamous and that protandry is the rule, although it varies in degree. In diploid varieties it is usually more strongly expressed than in triploids. In some varieties, such as Conference, a high proportion of the anthers dehisce in the bud stage before the petals open. In other varieties, e.g. Fertility, they dehisce one to two days after the petals open ; but even in such varieties the stigmas are not receptive until several days later. In triploid varieties the anthers dehisce about two days after the petals open and the stigmas are usually receptive on the day following dehiscence.

Fig. 8.
Length of pollen tubes in Conference selfed at 30° C., 24 hours after pollination.

The period of time between petal opening and dehiscence of the anthers varies, however, with meteorological conditions ; in most varieties the interval is longer under humid than under dry conditions.

The effect of the age of the flower at the time of pollination on pollen tube growth was investigated in Conference (2x) and in Beurré d'Amanlis (3x). Full stigmatic receptivity was noted in Beurré d'Amanlis on the third and in Conference on the fourth day after the opening of the flower. The petals of Beurré d'Amanlis fell off on the sixth day and those of Conference on the ninth day after opening. At this time the embryo-sacs of both varieties still appeared to be normal, no degeneration or shrivelling being evident. Fifteen days after opening of the flower the stigmas of Beurré d'Amanlis started to turn brown and the flowers began to fall. At this time the stigmatic surfaces of both varieties were still moist.

Self-pollinations were made on flowers of these two varieties on the day they opened and at twenty-four hours intervals up to fifteen days. These flowers were fixed twenty-four hours after pollination and incubation at 30° C.

The self pollen applied to the styles of Conference on the day the flowers opened entirely failed to germinate ; in Beurré d'Amanlis some germination occurred and tubes had begun to penetrate the styles. Flowers of the two varieties were also pollinated with Fertility (2x) on the days they opened. In the Conference styles crossed with Fertility a few pollen tubes had formed, reaching a length of 2·2 mm. Considerable pollen tube growth had taken place in the styles of Beurré d'Amanlis pollinated with Fertility on the day the petals opened, the longest tubes having reached the base of the style—about 8 mm.

With the age of the flower, the rate of pollen tube growth increased in both varieties when selfed ; in Conference it attained a maximum on the ninth day (Fig. 8). In fully receptive

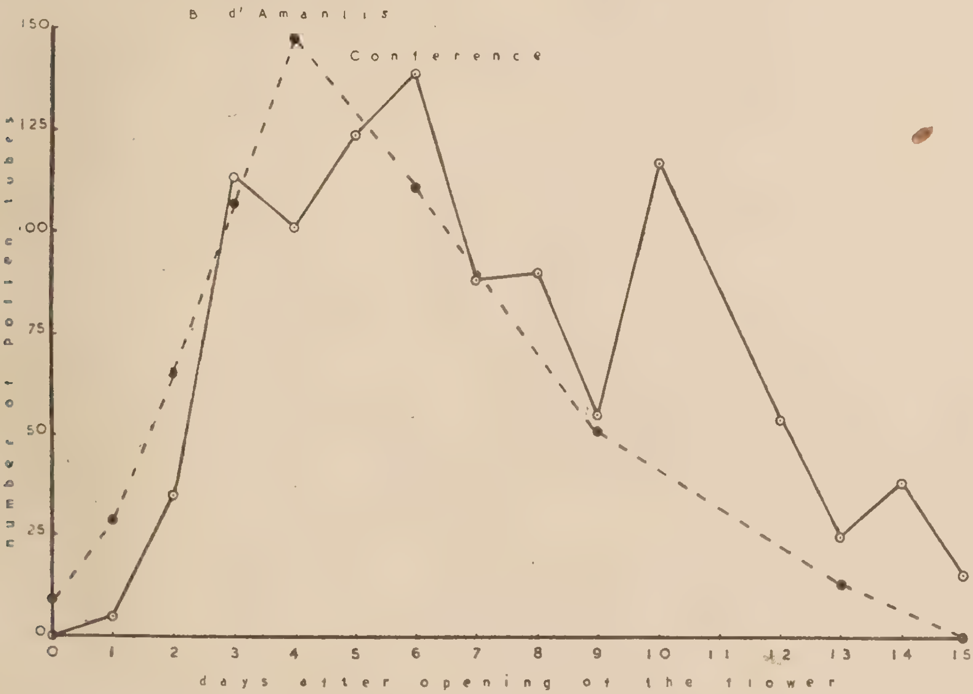

FIG. 9.
Number of pollen tubes per flower 24 hours after pollination at 30° C.

styles of Beurré d'Amanlis from the third day of opening onwards a few of the longest tubes reached the ovary in twenty-four hours.

The number of pollen tubes, growing down the styles of the flower was also related to the age of the flower. In both varieties the number of tubes was low in the early, pre-mature, and in the very late pollinations (Fig. 9). The same applies to the percentage of swollen-ended tubes in Beurré d'Amanlis. In the late pollinations of Conference, however, the proportion of swollen tubes did not decrease (Table X).

Stigmatic receptivity was not reached simultaneously by all the styles of a single flower and thus the period of receptivity of a single flower was prolonged. It was most pronounced in the 1-2 and 13-14 days old flowers, when germination took place on some of the stigmas but not on others. Thus, in the early flowers some stigmas had not reached the stage of receptivity while in other flowers some stigmas had passed it. Differences which I observed in the lengths of pollen tubes in different styles of the same flower were doubtless due to variations in the

TABLE X.

Conference selfed twenty-four hours after pollination at 30° C.

Age of flower in days.	No. of styles per flower.	Mean length of tubes.	Coefficient of variation.	No. of tubes per flower.	% sw. tubes.
			%		
Opening	5	0·0	0	0	0·0
1	5	0·5	93	5	0·0
2	5	1·4	31	35	42·8
3	5	1·5	15	113	29·2
4 full recept.	5	1·5	13	101	26·7
5	5	2·3	14	124	55·6
6	5	2·4	28	139	49·6
7	5	2·0	5	88	46·1
8	4	2·7	7	71	50·7
9 petal fall	3	4·0	3	33	33·3
10	5	2·8	4	117	36·0
12	5	2·2	36	54	46·3
13	5	2·2	29	25	60·0
14	4	0·7	79	38	39·5
15	5	1·4	98	21	42·9

TABLE XI.

Beurré d'Amanlis selfed twenty-four hours after pollination at 30° C.

Age of flower in days.	No. of styles per flower.	Mean length of tubes.	Coefficient of variation.	No. of tubes per flower.	% sw. tubes.
			%		
Opening	5	0·4	138	9	0·0
1	5	1·0	80	29	24·1
2	5	6·2	39	65	61·5
3 full recept.	5 ovary	5·7	39	107	53·1
4	5 ,,	5·3	11	148	57·4
6 petal fall	5 ,,	8·5	14	111	55·9
9	5 ,,	6·9	26	51	33·3
13	5	2·2	128	13	30·8
15	5	0·0	0	0	0·0

TABLE XII.

Fruit and seed setting in pears.

	No. of flowers.	No. of fruits.	% of fruits.	No. of seeds.	Seed per fruit.	% of seed per ovule.
Conference, selfed	5,313	41	0·7	57	1·4	0·11
Beurré d'Amanlis, selfed	1,562	7	0·5	1	0·1	0·05
Beurré d'Amanlis × Conference ..	523	0	0·0	0	0·0	0·00
Conference × Beurré d'Amanlis ..	845	25	3·0	33	1·3	0·39
Beurré d'Amanlis × Fertility	450	21	4·7	33	1·6	0·73
Fertility × Conference	431	23	5·3	208	9·0	4·83

receptivity of stigmas. This is shown by the coefficient of variation for the longest pollen tubes, which is fairly low in fully mature flowers (Tables X and XI ; Fig. 10).

The failure of fruit setting in pears on premature pollination is probably caused by the loss of viability of the pollen when in contact with an immature stigma. This was indicated by the lack of germination of the pollen remaining on the stigma for forty-eight hours. Hence in pears, it is unlikely that fruit will set after " bud pollination ", as has been observed in some other self-incompatible plants, e.g. *Brassica oleracea* (Kakizaki, 1930), Nicotiana *sp.* (East, 1934) and *Petunia violacea* (Yasuda, 1939).

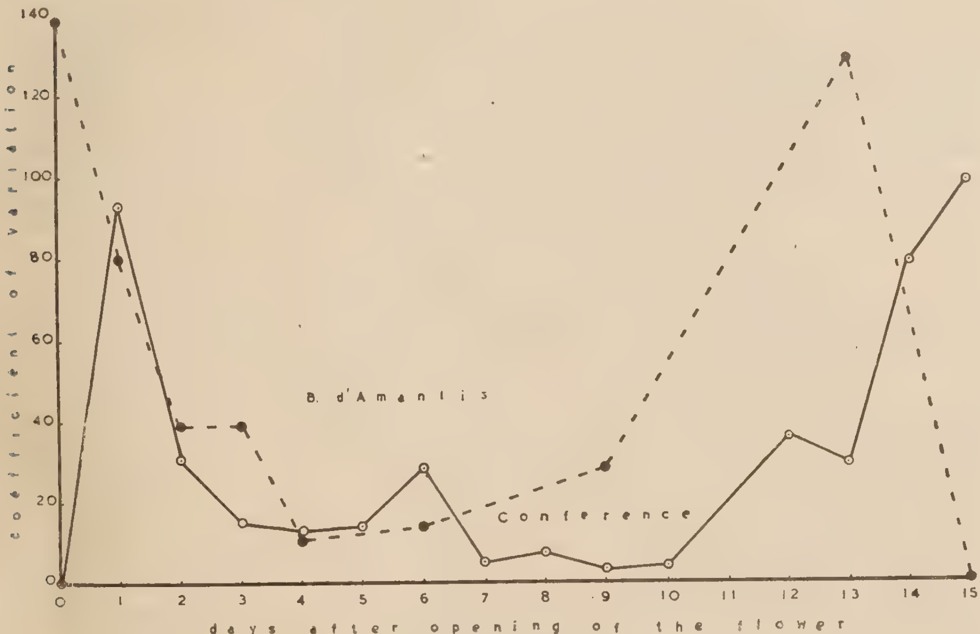

FIG. 10.

Coefficient of variation for the pollen tube length 24 hours after pollination at 30° C.

SELFING IN PEARS.

Conference (2x) is highly self-incompatible (Table XII). When self-pollinated, pollen tube growth is very slow and only very short tubes develop. In a period of twenty-four hours after pollination the maximum growth was obtained at a temperature of 20° C. (Table XIII ; Fig. 11), but forty-eight hours after pollination the maximum growth was obtained at 10° C., and it was only at this temperature that further growth and increase in pollen tube length took place (Table XIV).

In the triploid variety Beurré d'Amanlis when selfed the rate of pollen tube growth twenty-four hours after pollination was slightly lower at 10° C. than that of Conference, but it increased rapidly with rise in temperature, the tubes reaching the ovary at 30° C. in twenty-four hours (Table XIII ; Fig. 11).

During the next twenty-four hours the pollen tubes of Beurré d'Amanlis, unlike those of Conference, increased in length at all temperatures. At 10° C. they were still shorter than those of Conference, but surpassed them at higher temperatures. A proportion of the tubes remained

Table XIII.

Pollen tube growth in pears twenty-four hours after pollination.

Varieties.	Temperature.			
	10° C.	20° C.	25° C.	30° C.
Selfing :	mm.	mm.	mm.	mm.
Conference (2x)	1·8	2·4 (4)	2·0 (7)	2·0
Beurré d'Amanlis (3x)	1·5	4·1	8·1	ovary
Bartlett (4x)	3·1	5·7	ovary	,,
Crossing :				
Bartlett (4x) × Bartlett (2x)	2·2	4·4	3·8	3·9
Beurré d'Amanlis (3x) × Conference (2x)	3·1	4·3 (4)	3·8	3·6
Conference × Beurré d'Amanlis	1·2 (7)	2·9 (4)	3·7	2·7 (8)
Beurré d'Amanlis × Fertility (2x)	3·4	7·0	ovary	ovary
Fertility × Conference	2·5	7·1	,,	,,

Table XIV.

Pollen tube growth in pears forty-eight hours after pollination.

Varieties.	Temperature.			
	10° C.	20° C.	25° C.	30° C.
Selfing :				
Conference (2x)	3·9	1·9	2·4	1·7
Beurré d'Amanlis (3x)	2·3	7·4	ovary	ovary
Crossing :				
Beurré d'Amanlis (3x) × Conference (2x)	5·0	4·6	5·1	3·3
Conference × Beurré d'Amanlis		4·3	4·4	3·7
Beurré d'Amanlis × Fertility (2x)	7·4	ovary	ovary	ovary
Fertility × Conference	8·2	,,	,,	,,

Table XV.

Number of pollen tubes per style and per cent. of swollen-ended tubes twenty-four hours after pollination.

Varieties.	Temperature.							
	10° C.		20° C.		25° C.		30° C.	
	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.
Selfing :								
Conference (2x)	8·3	12·0	23·0	59·8	33·6	45·5	20·2	26·7
Beurré d'Amanlis (3x)	5·3	18·7	24·0	46·6	41·0	52·8	41·0	61·0
Bartlett (4x)	12·3	2·7	13·3	0·0	26·0	20·5	9·7	27·6
Crossing :								
Bartlett (4x) × Bartlett (2x)	10·0	3·3	20·3	14·8	24·3	43·8	15·0	35·6
Beurré d'Amanlis (3x) × Conference (2x)	18·3	1·8	44·2	54·8	57·3	50·6	83·7	61·0
Conference (2x) × Beurré d'Amanlis (3x)	6·1	0·0	23·0	30·4	11·7	31·4	11·9	37·9
Beurré d'Amanlis (3x) × Fertility (2x)	14·7	2·3	26·0	5·1	26·0	5·1	25·0	14·7
Fertility (2x) × Conference (2x)	10·7	3·1	13·3	4·9	17·0	31·4	22·7	17·6

in the style at 30° C. These were about the same length as those in Conference, thus showing their incompatible nature (Fig. 13).

The rate of pollen tube growth of the tetraploid Bartlett, twenty-four hours after selfing, was higher at all temperatures than in the above-mentioned diploid and triploid varieties. The pollen tubes were much longer at 10° C. and reached the ovary twenty-four hours after pollination (Table XIII ; Fig. 11). After the first twenty-four hours following pollination the number of pollen tubes per style was high, both in Conference and in Beurré d'Amanlis, but lower in the tetraploid Bartlett (Table XV). In general, fewer pollen tubes were found at 10° C.

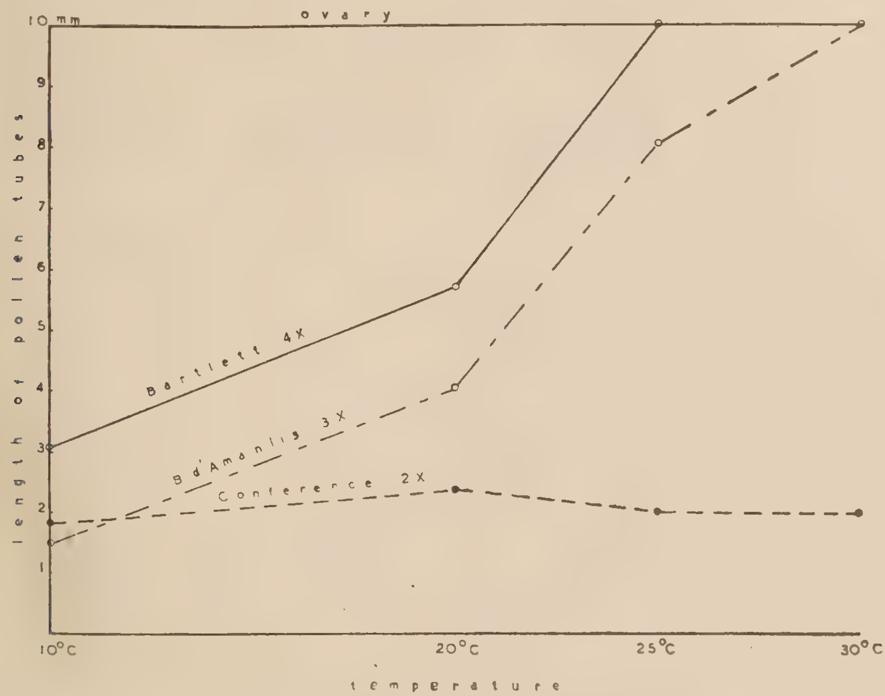

FIG. 11.
Pollen tube growth in self-pollinated pears 24 hours after pollination.

than at higher temperatures, because the higher temperatures are more favourable for pollen germination.

The percentage of incompatible tubes with swollen ends was high, both in Conference and Beurré d'Amanlis, especially at the higher temperatures (Tables XV and XVI ; Plate III, Fig. 7). Sometimes " forked " or " stubbed " tubes were found (Plate III, Fig. 8).

In Bartlett (4x), twenty-four hours after pollination, tubes with swollen ends appeared only at higher temperatures (25° C. and 30° C.).

The distribution of pollen tubes in the styles also differed in the varieties investigated. In selfed Conference, only the incompatible type was found, both twenty-four hours and forty-eight hours after pollination (Fig. 12). In Beurré d'Amanlis bimodality of pollen tubes was evident twenty-four hours after pollination (Fig. 13). At that time the majority of the tubes were short (2 mm.), as in Conference, the rest being distributed in the lower part of the style. The short tubes were inhibited in growth at higher temperatures because of their incompatibility reaction with the style. The long tubes, whose growth was accelerated by high temperature,

were compatible. These two types of tubes were most clearly defined forty-eight hours after pollination, when at 30° C. the compatible tubes had already reached the ovary while the incompatible tubes remained arrested in the upper part of the style, like those of Conference.

TABLE XVI.

Number of pollen tubes per style and per cent. of swollen-ended tubes forty-eight hours after pollination.

Varieties.	Temperature.							
	10° C.		20° C.		25° C.		30° C.	
	No.	% sw.	No.	% sw.	No.	% sw.	No.	% sw.
Selfing :								
Conference (2x)	27·0	48·1	34·0	66·7	26·0	60·3	7·0	33·3
Beurré d'Amanlis (3x)	9·7	34·5	13·0	43·6	20·0	48·3	7·3	36·4
Crossing :								
Beurré d'Amanlis (3x) × Conference (2x)	30·3	11·0	23·7	28·2	56·3	63·9	68·3	62·0
Conference (2x) × Beurré d'Amanlis (3x)			10·0	50·0	13·0	43·6	7·3	36·5
Beurré d'Amanlis (3x) × Fertility (2x)	25·7	33·8	24·3	38·4	13·7	7·3	9·0	3·7
Fertility (2x) × Conference (2x)	17·0	9·8	6·7	5·0	9·3	17·9	9·3	14·3

Two types of pollen tubes were also present in the tetraploid Bartlett : (1) short, incompatible tubes, (2) long, compatible tubes. This bimodality found in the triploid and tetraploid pears is in agreement with the hypothesis of Lewis and Modlibowska (1942) and Lewis (1943) explaining the breakdown of self-incompatibility in polyploids. The incompatible tubes would on this hypothesis carry two of the same (S) allelomorphs while the compatible tubes would carry two different (S) allelomorphs.

CROSSING IN PEARS.

The great majority of pear varieties are compatible when inter-crossed. So far only a few examples of cross-incompatibility have been found ; they are :

Belle Lucrative □ Louise Bonne de Jersey (Johansson and Callmar, 1936).
Belle Lucrative △ Seckel (Johansson and Callmar, 1936).
Louise Bonne de Jersey △ Seckel (Johansson and Callmar, 1936).
Louise Bonne de Jersey △ Williams' Bon Chrétien (Osterwalder, 1910).
Williams' Bon Chrétien △ Seckel (Marschal *et al.*, 1929, cited by Schanderl, 1937).
Beurré d'Amanlis (3x) ⊙ Conference (2x) (Crane and Lewis, 1942).
Fertility (4x) ⊙ Fertility (2x) (Crane and Lewis, 1942).

□ No record of reciprocal cross. △ Both ways incompatible. ⊙ One way incompatible.

The last two crosses are incompatible only in one direction, viz. when the high polyploid is used as the female. Bartlett (4x) × Bartlett (2x) are also incompatible (Lewis, unpublished).

The growth of the pollen tubes was investigated in the incompatible crosses Beurré d'Amanlis (3x) × Conference (2x) and Bartlett (4x) × Bartlett (2x), and compared with that of the compatible pollinations Beurré d'Amanlis (3x) × Fertility (2x), Fertility (2x) × Conference (2x) and Bartlett (4x) self-pollinated.

Twenty-four hours after pollination the lengths of the pollen tubes in the incompatible crosses when plotted formed a curve (Fig. 14) similar to that of Conference selfed (Fig. 11), with maximum growth at 20° C. In the compatible crosses, on the other hand, and in the Bartlett

(4x) selfed, the pollen tube growth rate increased rapidly with rise in temperature, maximum growth being obtained at 30° C.

Forty-eight hours after pollination little increase in pollen tube length took place in Beurré d'Amanlis × Conference at 10° C. to 25° C., and none at 30° C. (Table XIV). In the compatible pollinations—Beurré d'Amanlis × Fertility (2x), Fertility (2x) × Conference, and Bartlett (4x) selfed—the pollen tubes were still growing unchecked, and at a temperature of 20° C. many had reached the ovary by this time. The number of pollen tubes per style and the frequency of tubes with swollen ends was higher in Beurré d'Amanlis × Conference than in the compatible

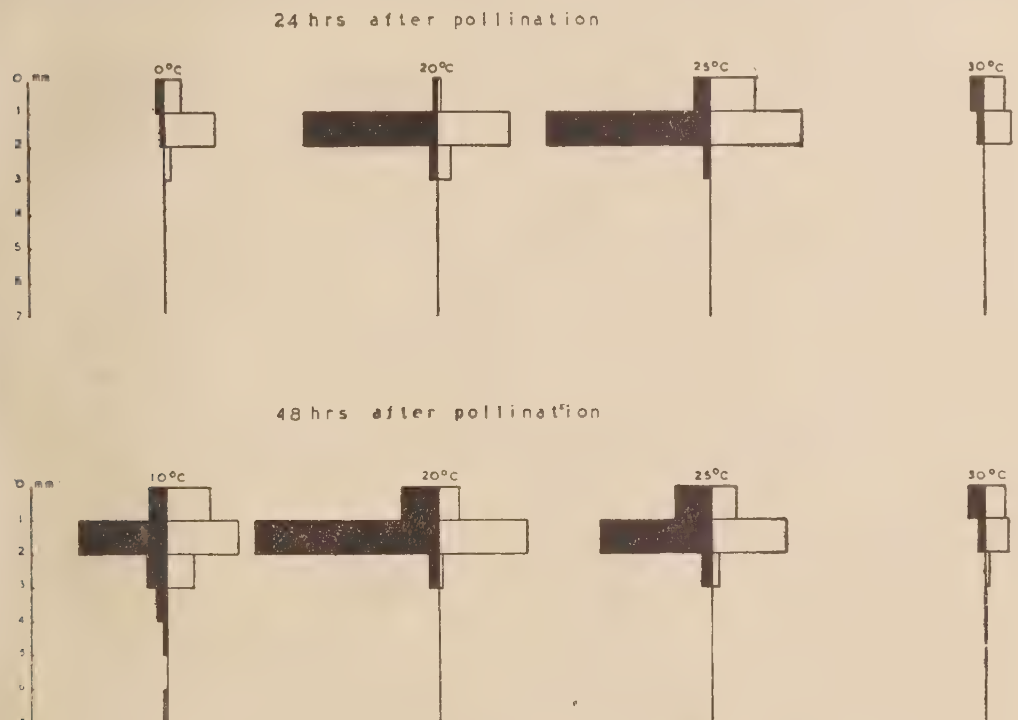

FIG. 12.
Distribution of pollen tubes in the styles of Conference selfed.
normal tubes ; swollen tubes.

crosses. There was a considerable difference between the two types of crosses, but a marked similarity among the crosses of each type.

It is evident that Beurré d'Amanlis carries genes which are incompatible with those of Conference ; in addition it has genes which are compatible with Conference. Hence seeds and fruits are formed when Conference is used as female in the reciprocal pollination. Many examples of one-way incompatibility have been reported in polyploid plants, but they are rare in diploids, and then usually in homozygous plants which have been deliberately produced (East, 1929).

Both incompatible and compatible tubes were seen in the styles of Conference when pollinated by Beurré d'Amanlis. At twenty-four hours the compatible tubes had reached their maximum length at a temperature of 25° C., whereas in the other pollinations the optimum temperature for maximum growth of compatible tubes at twenty-four hours was found to be 30° C. At forty-eight hours after pollination the number of pollen tubes in the styles was

approximately the same as in other compatible pollinations examined, but the proportion of tubes with swollen ends was much higher.

DISCUSSION.

Comparing the pollen tube growth of pears with that of apples a general similarity is evident. Thus there is :

(1) a similar type of incompatibility reaction ;
(2) an accelerating influence of high temperature on the growth of compatible tubes ;

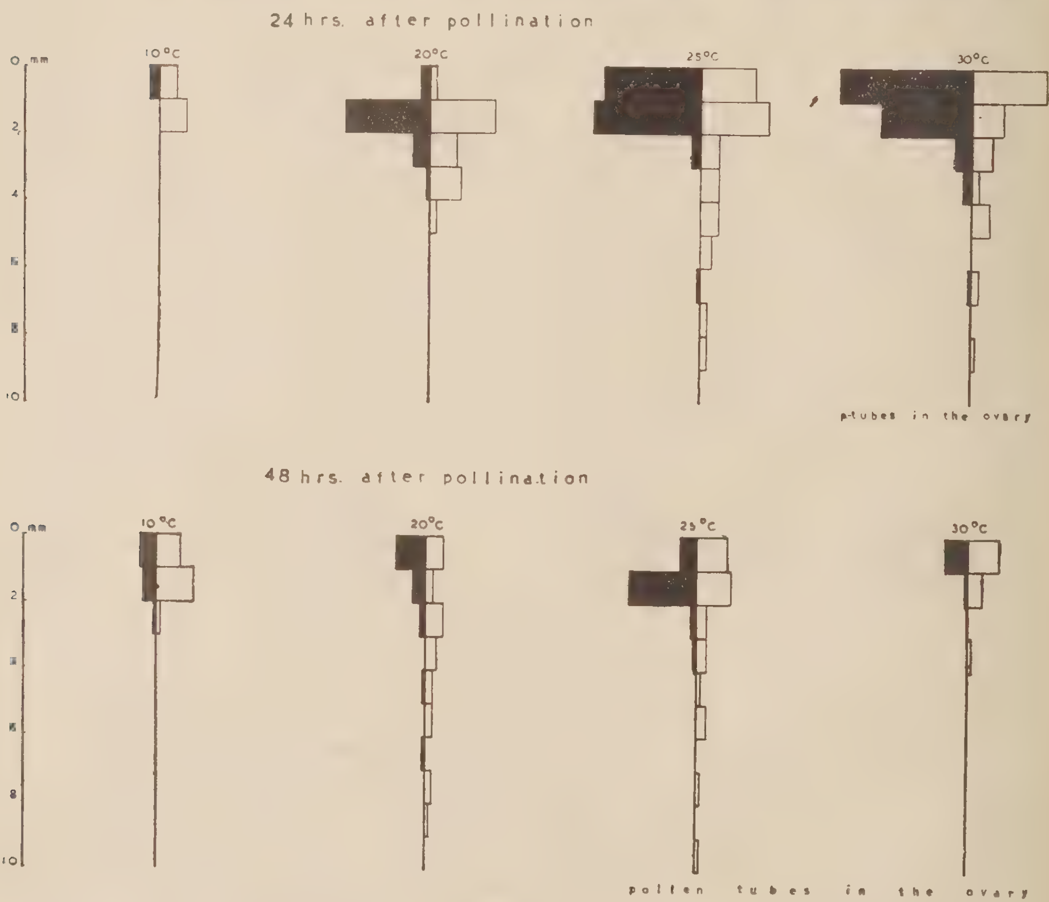

FIG. 13.
Distribution of pollen tubes in the styles of Beurré d'Amanlis selfed.
normal tubes ; swollen tubes.

(3) an inhibitory effect of high temperature on the growth of incompatible tubes ;
(4) a complete arrest of incompatible tubes in the style, the time and place of arrest depending on temperature ;
(5) a bimodality of pollen tubes in triploid varieties when self-pollinated ;
(6) an incompatibility reaction leading to the development of pollen tubes with swollen ends.

The main difference found between the pears and apples investigated here was that only one type of incompatible pollen tubes was seen in the selfed diploid pears, while in some apples bimodality was very pronounced. This is in agreement with the sharply defined self-incompatibility in pears as compared with the gradation of this condition in apples.

SUMMARY.

Incompatibility in apples and pears is due to physiological reactions occurring between the pollen tube and the stylar and ovarian tissue. In some cases the reaction takes place at an early stage, pollen tube growth being inhibited in the style, in others it takes place much

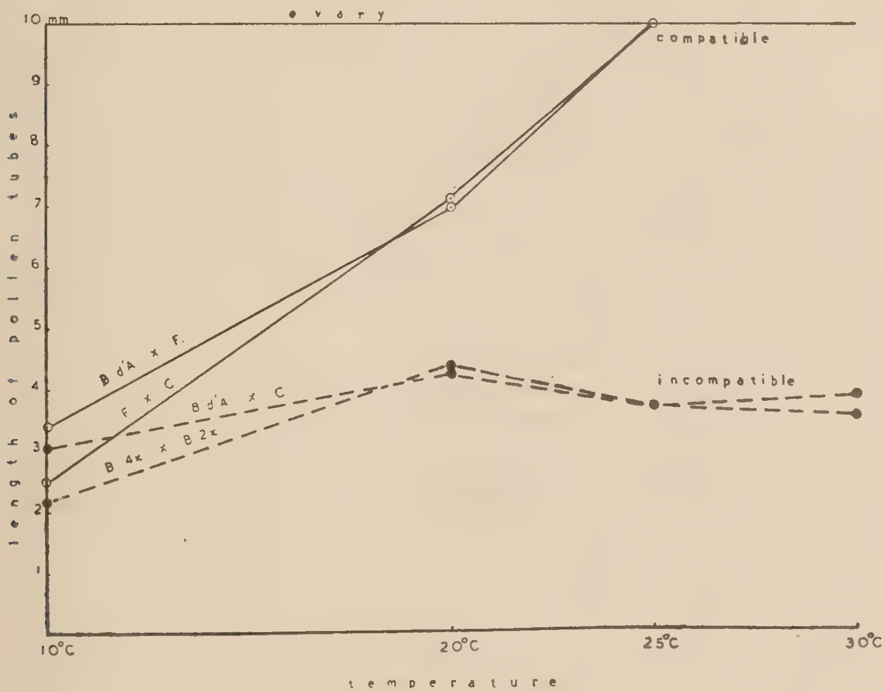

FIG. 14.

Pollen tube growth in cross-pollinated pears 24 hours after pollination.

later, even when the tubes have reached the ovarian tissue. These reactions are genetically controlled ; the essential feature of the genetic behaviour of incompatibility is that pollen cannot function in a style or ovary which carries the same incompatibility genes (usually designated as (S) genes) as the pollen.

TYPES OF POLLEN TUBES.

In apples and pears three well-defined classes of pollen tubes can be distinguished :

(1) *Incompatible tubes.*—These grow slowly and are inhibited at an earlier stage at a temperature of 25° C.-30° C. than at a lower temperature of 10° C.-20° C. At 25° C.-30° C. they are completely arrested in the upper part of the style and a high proportion of them develop swollen ends.

(2) *Semi-compatible tubes.*—These tubes also grow slowly, but unlike the completely incompatible tubes in Class 1, they do not become inhibited in the style. They continue to grow at all temperatures and reach the ovary, but seldom effect fertilization.

(3) *Compatible tubes.*—The rate of growth of these tubes at a low temperature (10° C.-15° C.) is similar to that of those in Classes 1 and 2, but it is more accelerated by a rise in temperature. Compatible tubes reach the ovary earlier than the semi-compatible tubes, and as a rule they effect fertilization.

Temperature, therefore, affects the growth of incompatible tubes in opposite ways. A high temperature speeds up the incompatibility reaction and this results in an early inhibition of the growth of incompatible tubes. On the other hand, compatible tubes are the most rapidly growing ones at all temperatures and their rate of growth is accelerated by a rise in temperature.

Self-pollination.

Diploids selfed.—In self-pollinated diploid apples both incompatible and semi-compatible tubes occur, while in pears only incompatible tubes were found. This is in agreement with the sharply-defined incompatibility in pears as compared with the gradation of this condition in apples, and also with the more sharply defined and discontinuous segregation of morphological characters in pears than in apples.

In self-pollinated apples some shrivelled ovules and degenerating embryo-sacs were found six days after pollination. This was followed by an early dropping of the fruitlets. In pears no degeneration was observed during this early period, and the dropping of fruitlets did not occur so early in pears as in apples.

Triploids selfed.—Self-incompatibility in triploid apples and pears is not so strongly expressed as in diploids. In all triploids both incompatible and compatible tubes were present. In some varieties fertilization and endosperm development were observed following self-pollination.

Tetraploids selfed.—In the tetraploid pear, somatically derived from a self-incompatible diploid variety, both incompatible and many compatible tubes were present. This supports the view that polyploidy can remove the bar to self-compatibility, and also the hypothesis that this is due to the occurrence and interaction of two different incompatibility genes in the pollen grain.

Cross-pollination.

Diploids × diploids.—Most pollinations between diploids are compatible, but some incompatible pollinations occur both in apples and pears. In compatible cross-pollinations the rate of pollen tube growth is much more rapid than in self-pollinations, particularly at higher temperatures, 25° C.-30° C. The pollen tubes reach the embryo-sac and effect fertilization in forty-eight hours after pollination. In some embryo-sacs several endosperm nuclei were present at this time. Proembryos were first seen six days after pollination. Some of the cross-pollinations are only partially compatible. This can be recognized by the presence of both compatible and incompatible tubes in the styles, thus indicating gametic control of the incompatibility reaction. In incompatible cross-pollinations the behaviour of the pollen tubes is essentially the same as in diploid self-pollinations.

Triploids × diploids.—In these pollinations both compatible and incompatible combinations occur. Incompatibility is relatively more frequent in triploid × diploid pollinations than in diploid × diploid pollinations. This is because the chance of the incompatibility genes of the pollen meeting similar genes in the style is increased with the rise in chromosome number of the female plant.

The pollen tube growth in compatible pollinations of this group approximates to that in compatible pollinations between diploids. The same holds true for the pollen tube growth in incompatible crosses ; it is similar in both groups, triploids × diploids and diploids × diploids.

Tetraploids × diploids.—The study in pears has shown that the pollen tubes of a diploid variety are incompatible with the styles of a tetraploid that has arisen by the doubling of the chromosome number of the pollen variety.

Diploids × triploids.—No completely incompatible pollinations were found in this group of crosses. The low fertility in these pollinations is due mainly to high pollen sterility, which obscures the role of incompatibility. The pollen tubes of triploid apples, owing to their greater diameter, grow more slowly and effect fertilization later than those of diploids.

Triploids × triploids.—This group of pollinations gives results very similar to those obtained from diploid × triploid pollinations ; pollen tube growth is slow, fertilization late and fertility low.

The rate of growth of compatible pollen tubes in apples is approximately the same in diploid and triploid styles, but it differs according to the chromosome number of the pollen parent.

ACKNOWLEDGMENTS.

I wish to express my grateful thanks to Mr. M. B. Crane and Dr. D. Lewis for their interest, helpful criticism and advice throughout the course of the work, and to Mr. Crane also for access to the results of his extensive experiments.

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PLATE I.

(×230)

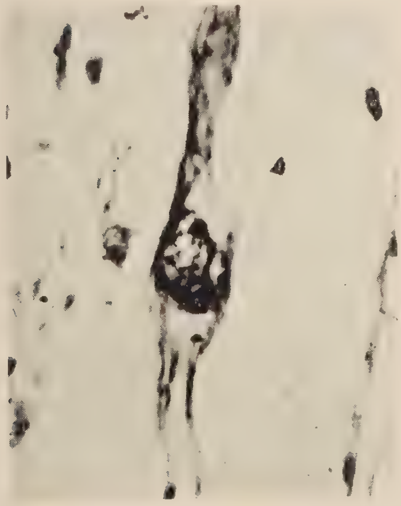
A

(×230)

B

Fig. 1.

Ends of apple pollen tubes in self-pollinations showing a difference in the swelling. A, at 15° C., B, at 30° C. 48 hours after pollination.



(×410)

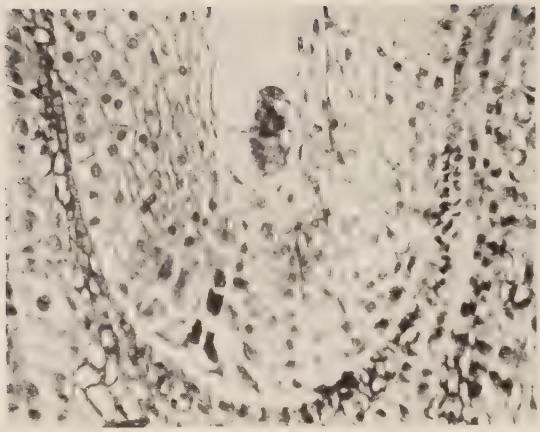
Fig. 2.

Triple fusion in Bramley's Seedling 6 days after pollination.

(×230)

Fig. 3.

Embryo-sac of Blenheim Orange showing that fertilization has not occurred 6 days after pollination.



(×230)

FIG. 4.

Proembryo of Bramley's Seedling ×Cox's Orange 12 days after pollination.

(×96)

FIG. 5.

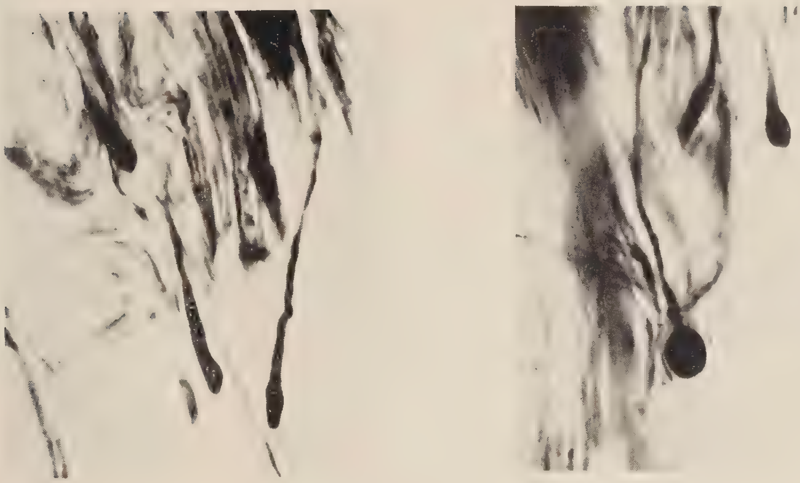
Shrinking ovule of Cox's Orange × Bramley's Seedling 48 hours after pollination at 25° C.

(×96)

FIG. 6.

Developing ovule with zygote and endosperm nuclei in Cox's Orange ×Baumann's Reinette 48 hours after pollination at 25° C.

PLATE III.

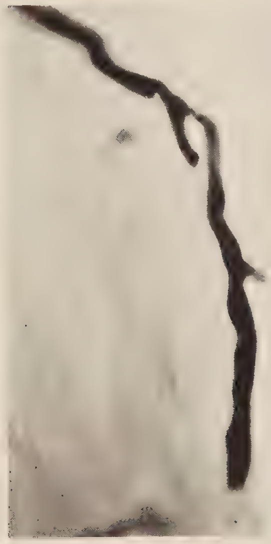


(×230) (×230)

A B

Fig. 7.

Swollen ends of pollen tubes in pears 24 hours after self-pollination.



(×460)

Fig. 8.

"Forked" pollen tube of pear 24 hours after pollination.

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STUDIES IN THE DIAGNOSIS OF MINERAL DEFICIENCY

II. A COMPARISON OF THE MINERAL CONTENT OF SCORCHED AND HEALTHY LEAVES FROM THE SAME APPLE TREE

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It is well known that an inadequate supply of certain nutrient elements leads to the appearance in the leaves of fruit trees of symptoms usually described as "scorch". In 1922 a type of leaf scorch in the apple associated with potassium deficiency was described (Wallace, 1922; Barker *et al.*, 1922), and later Wallace (1925) described another type due to magnesium deficiency. Scorch is, however, not always a nutritional phenomenon; it may be caused by wind (Barker and Gimingham, 1914), and injury by spray fluids often produces similar results. The cause of scorch in any given case can often be identified with some confidence from the distribution of the scorched areas on the leaf and the incidence of defoliation on the tree. These characters are, however, subject to considerable varietal differences (Wallace, 1929), and cases are not infrequent where it appears desirable to supplement visual diagnosis of the disorder as a nutrient deficiency by one based on plant analysis.

It is then necessary to consider the question whether it is desirable to sample scorched leaves or apparently healthy ones—for it is rare to find a tree with no apparently healthy leaves. Some of the investigators who have been faced with this problem have preferred to collect leaves normal in appearance. For instance, Reuther and Boynton (1940) as far as possible avoided selecting necrotic leaves; Drosdoff and Painter (1942) avoided badly necrotic leaves; and Wallace (1943) considered that "the leaves utilized should be metabolically active when sampled," though he was prepared to accept leaves showing deficiency symptoms to a certain extent. In an earlier investigation (1939), however, Wallace used leaf samples which were "as far as possible, showing the typical magnesium-deficiency breakdown symptoms". This general preference for apparently normal leaves has usually been based on *a priori* considerations—that the manifestly abnormal metabolism of scorched leaves would be liable to lead to changes in their mineral composition which, consequently, would give a false impression of the nutritional status of the tree. In particular it has been suggested that a scorched leaf is in many respects similar to a leaf undergoing normal senescence (Hardy, 1937); and many investigators (Combes and Échevin, 1926; Deleano and Andreesco, 1932; Gäumann, 1935) have shown that a substantial proportion of the nutrient elements present in the senescent leaf becomes translocated into the stem before abscission. On the other hand, it would appear reasonable to suppose that whether or not a particular leaf exhibits scorching depends on the concentration of the deficient element in that leaf, and therefore that a sample of scorched leaves should show a wider difference from the normal concentration of that element than a sample of healthy leaves from the same tree.

It is evident that this question cannot be settled without experimental evidence, and this is very scanty. Among the few observations bearing directly on the subject are those of Southwick (1943) who compared healthy and scorched leaves from a single apple tree suffering from magnesium deficiency, and found the magnesium content lower in those showing scorch. The leaves were not, however, fully comparable morphologically, and the difference in magnesium content may perhaps be ascribable to the morphological difference. Warne (1934) compared the healthy parts of pear leaves suffering from apical blackening with normal leaves of the same tree and found the potassium content of the former to be rather higher. In *Iris germanica*,

Helianthus multiflorus, *Aralia edulis* and *Allium cepa* he found differences in the opposite direction, but in these plants morphological factors were also involved. Oserkowsky and Thomas (1933) found no difference in the copper content of pear leaves suffering from exanthema (a disease ascribed to copper deficiency) and apparently healthy leaves from the same tree. Reuther and Boynton (1940) compared normal apple leaves with necrotic leaves from the same tree in instances where the necrosis was due to spray injury, excessive applications of cyanamide, potassium deficiency, and flooding. In each case the potassium content was lower in the affected leaves, but where necrosis was not due to potassium deficiency the potassium content of the affected leaves was nevertheless well within the normal range. Boresch (1938) analysed currant leaves suffering from marginal leaf scorch and healthy leaves from the same bush. The potassium content was lower and the chloride and total iron concentrations were higher in the former, but there was less water-soluble iron in them than in the healthy leaves. It is not clear, however, that the samples consisted of leaves comparable in age and morphological position. The same applies to the observations of Hardy (1937) on marginal leaf scorch of cacao; he found the potassium content of scorched leaves to be considerably lower than that of healthy leaves from the same tree.

Wallace and Mann (1926) and Wallace (1928), in investigations on lime-induced chlorosis in the apple, analysed separately chlorotic and normal leaves from the same tree. They found in the dry matter of the former high concentrations of potassium, phosphorus and usually ash, whilst calcium, and usually magnesium and iron, were low; but Wallace did not publish similar observations in his work on potassium and magnesium deficiency. Parbery (1935) and Fudge (1939) found that citrus leaves suffering from chlorosis due to magnesium deficiency contained less magnesium than healthy leaves on the same tree. However, Fudge showed that the chlorosis he investigated was due to the heavy demands on the plant's magnesium during fruit development, and it did not occur in the leaves on the non-fruiting (or lightly fruiting) branches from which the healthy leaf samples were taken. McGeorge (1939) investigated a type of chlorosis in grapefruit, the cure of which by sulphur application resulted in an increased manganese content of fruit and leaves. He found that the manganese content of chlorotic leaves was lower than that of green leaves from the same tree.

What little information is available, then, suggests that in a fruit tree with deficiency symptoms, the leaves actually showing symptoms contain rather less of the element in question than healthy leaves of the same tree, and from this point of view would be more informative for diagnostic purposes. However, there appears to be practically no information as to whether scorched leaves differ from healthy leaves of the same tree in the percentages (in the dry matter) of elements other than those causing the scorch, and whether there might consequently be a danger of false diagnosis if scorched leaves were sampled. To provide such information was the primary purpose of the investigation now to be described.

DESCRIPTION OF EXPERIMENTAL TREES.

A number of apple trees in the East Malling Research Station plantations were selected as having in the past regularly shown leaf scorch. At each location two similar trees growing side by side were sampled separately, to provide replication. Of the trees sampled, some had shown leaf scorch of the marginal type, ascribed to potassium deficiency; others showed interveinal scorch, usually associated with magnesium deficiency; while still others could not be placed definitely in either of these groups.

Showing marginal scorch:

A_1 and A_2. Lord Derby trees on Malling rootstock V, included in a pruning trial (Plot 4) planted in 1914 (Hatton and Grubb, 1925). These trees had been subject to severe marginal leaf scorch for a number of years (first recorded in 1919), and scorching had already started by the beginning of July 1942 when the first samples were taken.

B_1 and B_2. Lane's Prince Albert trees on Malling rootstock V, in a rootstock trial (Plot 11) planted in 1920 (Hatton, 1927). These trees had shown scorch, mainly of the marginal type, for some years.

C_1 and C_2. Egremont Russet trees on Malling rootstock V, in a variety trial (Plot 33) planted in 1921, and mentioned by Hatton and Hoblyn (1940). They had suffered from marginal leaf scorch for a considerable time.

Showing interveinal scorch.

D_1 and D_2. Lane's Prince Albert trees on Malling rootstock VII, in the same rootstock trial as B. These trees had shown scorch of the interveinal type for some years.

E_1 and E_2. Bramley's Seedling trees on Malling rootstock I. These trees were originally planted in 1920 as part of a rootstock trial (Plot 14), but from 1931 onwards differential manurial treatments were introduced. The experiment has been described by Wallace (1939) and Hoblyn (1941). The two trees in question received no fertilizer from 1920 to 1934. During the next five years they annually received, respectively, 8 and 4 cwt. of sulphate of potash per acre. No further applications were made to E_1 after 1939, while the treatment given to E_2 continued. Up to 1934 the trees had shown some marginal scorch, and since 1936 interveinal scorch had been severe in some seasons.

F_1 and F_2. Bramley's Seedling trees on Malling rootstock IV. These trees were in the same trial as E, and both trees received annually 8 cwt. of sulphate of potash per acre from 1931 to 1934, and thereafter half that quantity. Interveinal leaf scorch was present from 1936 onward.

G_1 and G_2. Bramley's Seedling trees on Malling rootstock VII. These trees were in the same trial as E and F, but received 8 cwt. of sulphate of potash per acre annually between 1934 and 1939 only. Their leaf scorch history was similar to that of E and F.

H_1 and H_2. Cox's Orange Pippin on Malling rootstock I, in a rootstock trial (Plot 32) planted in 1922 (Hatton, 1935). Some scorch, blotch and early defoliation had been observed on these trees since 1935, and they were stunted in growth.

I_1 and I_2. Worcester Pearmain trees in the same rootstock trial, which had been frameworked in 1932 on Stirling Castle on Malling rootstock V. Already in 1934 the foliage of these trees was of a poor colour, and in subsequent years it showed a certain amount of scorch.

J_1 and J_2. Rev. W. Wilks on Malling rootstock V, in the same variety trial as C. These trees had shown interveinal scorch consistently for a number of years.

Showing scorch of undefined type.

K_1 and K_2. Grenadier trees on Northern Spy rootstocks. These trees were in a rootstock trial planted in 1923 on the same plot as that including B and D. They had been subject for some years to fairly severe scorch.

L_1 and L_2. Egremont Russet trees in the same variety trial as C and J, topworked in 1940 on Bascombe's Mystery on Malling rootstock V. In the following year and in 1942 they showed considerable scorching of the foliage.

Methods of sampling and analysis.

The first leaf samples from the trees detailed in the previous section were taken between July 1st and July 3rd, 1942. At this time, the foliage of trees A was moderately scorched; very slight scorch was apparent in B_2, C_1, D_1, D_2, E_1, E_2, F_1, F_2, G_1, J_1, K_1, K_2, L_1 and L_2, but no scorch occurred on the rest of the trees sampled. The morphological type of leaf taken varied from tree to tree, according to the indications provided by the records as to which type was most likely to show scorch later in the season. Leaves from the centre of non-fruiting spurs were taken as samples from trees A, B and K ; from B and K separate samples were also taken from the centre of the long shoots. Leaves from the base of the long shoots were taken from

trees C, D, E, F, G, I and L; and from trees E separate samples were also taken from the base of the vegetative spurs. This latter type was the only one sampled in trees H and J. Each sample on this occasion consisted of ten healthy leaves.

Concurrently with the taking of these samples, fifty other leaves of the same morphological types on each tree were marked for future sampling by a length of thread tied loosely around the petiole. The purpose of this was to ensure that subsequent samples should be morphologically fully comparable with the first and with each other.

The second set of samples was taken on August 12th. Of the leaves marked with thread on each tree, alternate ones were taken and were subsequently separated into those showing and not showing scorch. A few of the threads had fallen, so that the total number of leaves in each sample averaged only 18, of which 6 were scorched to greater or less degree. The third set of samples, taken between October 7th and October 12th, consisted of all the remaining marked leaves, averaging 10 per tree, of which nearly half were scorched. For each sample of scorched leaves the range of intensity of the symptoms was noted.

In 1943 a single set of samples was taken from some of the same trees, namely B, C, E, G, I and L. The leaf types sampled were the same as in 1942, except that in E only the vegetative spur leaves were taken, and in B additional samples were taken from the central region of the secondary shoots from fruiting spurs. Where some of the leaves were severely scorched they were kept separate from those more slightly scorched.

The leaves sampled were cleansed by wiping with muslin, and the laminae were separated from the midribs; in 1942 the latter were discarded but in 1943 the petioles with midribs were analysed separately from the laminae. The samples were dried and ground in an agate mortar, and were then analysed spectrographically by a method differing little from that used previously (Goodall, 1943); a detailed description of the method is now being prepared for publication. All analyses were in duplicate, and the results are expressed on a basis of oven-dry weight.

In 1942, the elements for which data were obtained were calcium, iron, magnesium, manganese and potassium, while in 1943 sodium was added to the list. It should be pointed out that, owing to improvements in the methods between the times when the 1942 and the 1943 sets of samples were analysed, the analytical error was substantially lower for the latter series than for the former.

Unfortunately a certain number of the second set of samples in 1942 were burnt in the drying oven, with the consequence that these samples no longer form a coherent whole. The results have therefore been analysed and presented in several portions.

Experimental results.

In Table I are given for each pair of trees the content in the leaf laminae of the elements for which analyses were carried out, averaged over all samples irrespective of leaf type or condition or date of sampling (except that the more severely scorched samples of 1943 were omitted).

When the results of the analyses were examined, it was evident that the foliage of the six trees (A, B and C) which had been showing marginal scorch contained much less potassium than that of the others. The concentration of potassium in the leaves of these trees was barely half that in the other trees—the mean values being respectively, 0·45, 0·93 and 0·69% of dry matter, which may be compared with the limiting value of 1·00% K_2O (≡0·83% K) given by Wallace (1940, 1943) and the value of 1·00% K given by Reuther and Boynton (1940) as the level below which deficiency effects may be expected. On the other hand, Boynton (1942) and Batjer and Magness (1939) quote lower limiting values for the appearance of deficiency symptoms (0·75%, and 0·70% K respectively), so it may be regarded as open to some doubt whether the potassium content of B was low enough to account for the leaf scorch.

Most of the trees showing symptoms of interveinal scorch (D-J) had a magnesium concentration in the leaves considerably below the level of 0·40% MgO (≡0·24% Mg) indicated by Wallace (1939, 1940, 1940a, 1943) as the point below which magnesium-deficiency scorch may be observed, and the critical level of 0·25% Mg suggested by Southwick (1943). The same was true of the trees (K and L) showing symptoms that had not been referred to either definite category of scorch. Only on trees corresponding to F and G had the effect of magnesium treatment been tried; here some reduction in the intensity of interveinal scorch followed repeated applications of magnesium sulphate to the soil. The two trees H seem to be an anomalous case, since although they had shown considerable interveinal scorch the magnesium concentration in the leaves was well above the critical level, and the mineral composition of the foliage does not seem to supply any explanation of the scorch symptoms.

TABLE I.

Mean mineral content of all lamina samples from each situation.

Situation.	Number of Samples.*	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)	Sodium* (p.p.m.)
A	9	1·87	151	0·254	46·2	0·45	—
B	34	1·66	186	0·289	57·9	0·93	197
C	14	1·58	146	0·191	41·0	0·69	179
D	9	1·75	168	0·184	50·9	1·67	—
E	23	1·67	198	0·138	62·6	2·04	218
F	9	1·56	164	0·175	51·1	1·82	—
G	16	1·34	146	0·149	52·3	2·00	118
H	11	1·93	267	0·277	66·8	1·71	—
I	23	1·70	198	0·143	59·0	1·82	171
J	9	2·03	172	0·153	54·9	1·79	—
K	17	1·62	124	0·180	51·6	1·53	—
L	13	1·57	129	0·138	42·3	1·67	139

* The sodium determinations were carried out only on the samples collected in 1943, hence the numbers are lower than for the other elements, viz. 12 for B, 4 for each of the other situations.

The contrast between trees C and L is of considerable interest. The rootstock and scion variety were in both cases the same, but in L they were separated by trunks of Bascombe's Mystery, and the Egremont Russet portion was much smaller than in C. C shows fairly severe potassium deficiency, and a magnesium content which, though below normal, is not as low as in any of the other pairs of trees deficient in magnesium; L has a normal potassium content, but the lowest magnesium content found. The differences in the other elements are not such as to excite comment; the differences in potassium and magnesium content are statistically significant (although only four trees are involved). These facts suggest that the soil on which these trees are growing is deficient in both potassium and magnesium, the former deficiency being accentuated by the rootstock (Hatton and Grubb, 1925); that a larger proportion of the potassium than of the magnesium in the leaves was translocated into the trunk and larger boughs before leaf fall; and that thus an adequate amount of potassium was available to supply the needs of the reduced volume of foliage after top-grafting, whereas the magnesium supply was not sufficient. The data of Wallace (1929) indicate that the ratio of potassium to magnesium in apple shoots and trunks is higher than in the leaves; and, while the whole of these reserves is not necessarily available for leaf production, it is probable that the proportion of the potassium present which can be translocated to the growing points is higher than that of the less mobile magnesium.

There is no indication in these data that trees suffering from magnesium and from potassium deficiency differ consistently in the concentration in their foliage of any of the four other elements investigated.

Differences between scorched and healthy leaves.

Table II shows separately the mineral content of the scorched and healthy laminae of leaves from the same trees. The three occasions on which such samples were taken are shown separately; the potassium-deficient trees (A, B and C) and the magnesium-deficient trees (D, E, F, G, I, J, K and L) are also separated, a third series of values being supplied by the anomalous pair of trees H. Not all situations or leaf types are, of course, represented at each date of sampling. Of the 1943 samples, the more severely scorched leaves are excluded from this table.

TABLE II.

Mean mineral content of scorched and healthy laminae.

Situations.	Month of sampling.	Condition of leaves.	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)	Sodium (p.p.m.)
A, B, C ..	Aug., 1942	Healthy	1·76	107	0·192	41·8	0·66	—
		Scorched	1·77	132	0·298	40·5	0·68	—
A, B, C ..	Oct., 1942	Healthy	1·89	158	0·239	44·3	0·69	—
		Scorched	1·87	167	0·218	43·9	0·51	—
B, C	Sept., 1943	Healthy	1·38	140	0·270	63·0	0·85	164
		Scorched	1·39	205	0·248	64·1	0·83	187
D, E, F ..	Aug., 1942	Healthy	1·77	145	0·189	53·5	1·88	—
G, I, J, L ..		Scorched	1·70	141	0·163	55·0	1·75	—
D, E, F, G ..	Oct., 1942	Healthy	1·67	156	0·141	48·5	1·66	—
I, J, K, L ..		Scorched	1·76	157	0·115	43·1	1·53	—
E, G ..	Sept., 1943	Healthy	1·29	207	0·108	65·9	2·20	166
I, L		Scorched	1·34	191	0·078	63·3	2·15	141
H	Oct., 1942	Healthy	2·16	358	0·291	86·0	1·97	—
		Scorched	1·84	160	0·156	55·0	1·43	—

The results for each sampling date have been analysed statistically. Since the differences among situations were large in comparison with the means, it was necessary first to transform the data. A logarithmic transformation was selected, since previous observations had indicated its suitability, and the significance of proportional differences is of interest from the point of view of diagnosis. This procedure has also been followed in all the analyses described below, and in each case the means given are those obtained from the transformed data—i.e. they are geometric, not arithmetic means. The differences in composition between healthy and scorched laminae were found to be non-significant with the exception of the magnesium content. In the August 1942 samples, though the mean effect of scorch on magnesium content is not significant its interaction with deficiency (i.e. with the difference between the potassium-deficient and the magnesium-deficient groups of trees) just reaches the 5% significance level ($F=4\cdot702$, $n_1=1$, $n_2=13$). In the October 1942 samples, the mean effect of scorch approaches significance ($F=3\cdot163$, $n_1=1$, $n_2=21$), and in the 1943 sample set it passes the 5% significance level ($F=5\cdot144$, $n_1=1$, $n_2=10$); but in these sets the interaction with deficiency is non-significant. It thus appears that under conditions of magnesium deficiency, scorched leaves are likely to contain less magnesium than healthy leaves on the same tree, but that where potassium deficiency occurs, the magnesium content of the scorched leaves may on occasion be higher than that of adjacent healthy leaves. There is no indication in these data of any migration of the other mineral elements from the leaves as a result of their necrotic condition, nor any suggestion that an incorrect diagnosis would result from the sampling of scorched rather than healthy leaves.

In fact, the data might be taken to support the sampling of scorched leaves for confirming a diagnosis of magnesium deficiency.

For each sampling date, those leaf samples in which the degree of scorch was more severe (more than 10% of the area of the leaf affected) were considered separately, since it was possible that changes in mineral composition might occur only when the processes occurring in the leaf had reached a more advanced stage. In the 1942 sample sets, these samples again showed no significant general effects of scorch on composition.

For the five trees from which samples of leaves showing severe scorch were obtained in 1943, analyses are given in Table III. The mean effect of scorch on the magnesium content is significant ($F=6{\cdot}085$, $n_1=2$, $n_2=6$) and so is the effect if the potassium-deficient trees are considered alone ($F=30{\cdot}515$, $n_1=2$, $n_2=2$). Thus, when scorch due to potassium deficiency becomes severe, magnesium migrates from the leaves, and if such leaves had been sampled for diagnosis the false conclusion might have been drawn that the symptoms were due to a combination of potassium and magnesium deficiency. There is also a highly significant effect of severe scorch on the manganese content ($F=15{\cdot}404$, $n_1=2$, $n_2=6$), indicating that manganese, too, may be migrating from these leaves. Increased leaching by rain is another possible explanation.

TABLE III.

Mean mineral content of laminae showing different degrees of scorch.

Trees.	Degree of scorch.	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)	Sodium (p.p.m.)
B_2 and C_1 ..	None	1·36	179	0·319	60·0	0·71	206
	Slight	1·33	213	0·262	56·9	0·71	197
	Severe	1·41	221	0·160	45·1	0·55	261
G_1, G_2 and I_2 ..	None	0·99	190	0·104	69·0	2·35	130
	Slight	1·33	207	0·084	71·1	2·45	136
	Severe	1·04	189	0·057	48·1	2·64	177

Seasonal change in mineral composition of healthy leaves.

In addition to the main purpose of this investigation—the comparison of scorched and healthy leaves sampled from the same tree at the same time—the 1942 sets of samples also provide information on changes in the mineral composition of the leaves during the growing season. The relevant data are given in Table IV.

TABLE IV.

Mean mineral content of healthy laminae at different dates in 1942.

Situations.	Month	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)
A, B, C	July	1·61	100	0·182	41·9	0·63
	August	1·75	107	0·191	41·8	0·66
	October	1·89	173	0·239	44·3	0·71
D, E, F, G, I, J, K, L	July	1·52	127	0·178	49·0	1·67
	August	1·72	131	0·179	51·3	1·74
	October	1·69	148	0·140	46·6	1·63
H	July	1·67	204	0·211	41·1	1·46
	August	1·95	275	0·237	71·0	1·73
	October	2·16	358	0·291	86·0	1·97

The mean effect of date of sampling is significant only for calcium ($F=6\cdot356$, $n_1=2$, $n_2=41$); the seasonal increase agrees with the findings of Larson (1933) and Wallace (1939, 1940, 1943) in the apple, and of Combes and Échevin (1926), Sampson and Samisch (1935), Mitchell (1936) and Lilleland and Brown (1939), working with other deciduous trees. Iron, too, shows a seasonal increase, though this does not reach significance ($F=2\cdot405$, $n_1=2$, $n_2=41$); in elder and chestnut, Hill and Lehmann (1941) found a considerable decrease in the iron content of the leaves during the latter part of the summer. In the magnesium figures there is a highly significant interaction between date of sampling and deficiency ($F=5\cdot875$, $n_1=2$, $n_2=41$), the magnesium-deficient trees showing a decline in magnesium content between July and October, the rest an increase. The latter is in agreement with the results of Wallace (1939, 1940, 1943) in the apple, and of Lilleland and Brown (1939) in the plum; but there seem to be no earlier observations on seasonal changes in the magnesium content of the foliage of trees deficient in magnesium. The differences in manganese are non-significant, in agreement with the observations of Epstein and Lilleland (1942) who found little change between May and October in the manganese content of peach leaves. The potassium contents also show no significant differences, which is rather surprising in view of the numerous observations recorded in the literature of decreases in the potassium content through the season (Larson, 1933; Wallace, 1939, 1940, 1943; Batjer and Magness, 1939; Reuther and Boynton, 1940—to quote only observations on apple leaves). However, the principal decrease generally occurs from October onwards, when yellowing has begun; and very probably this stage had not been reached by the time the last set of samples was taken. Chandler (1936) found some increase in the potassium content between July and October.

From the point of view of deficiency diagnosis by plant analysis, the most interesting observation in these seasonal changes is that on magnesium. The difference between the magnesium content of leaves of trees suffering from magnesium deficiency and others not suffering from it increases markedly between July and October, which indicates that samples for diagnosis of this condition should be taken fairly late in the season. This is not in agreement with the view of McCollam (1943) that samples taken late in the season are likely to be uniformly low in mineral content, instead of showing differences in nutritional status.

It would, of course, be preferable to base deductions as to the comparative merits of different methods of deficiency diagnosis on comparisons between otherwise comparable plants known to show different yield responses to the nutrient in question. With fruit trees, however, this approach is rarely practicable, and it is generally necessary, as above, to fall back on comparison of proportionate differences in trees containing the element in question in different concentrations.

Comparison of different types of leaf.

The data obtained also provide material by which the observations made earlier on differences between different morphological types of leaf (Goodall, 1943) may be confirmed. In 1942, samples were taken from both the long shoots and the non-fruiting spurs of certain trees, either the basal or the middle leaves of these types of growth being sampled in each case.

In Table V are given comparisons of the middle leaves in July and October 1942, for the two pairs of trees from which they were collected.

The higher calcium content in the spur leaves agrees with the earlier observations. In the previous series the iron and manganese content of the spur leaves was somewhat higher than that of the shoot leaves, but here no significant differences occur. The higher magnesium content in the spur leaves is fully in line with the results reported in the earlier paper, but in the present series of samples the difference does not reach significance. With potassium, on the other hand, there is a highly significant interaction ($F=21\cdot860$, $n_1=1$, $n_2=6$) between leaf type and situation, the potassium content being higher in the shoot leaves of the potassium-deficient trees B, but higher in the spur leaves of the magnesium-deficient trees K. This is very satisfactory confirmation of the conclusion in the earlier paper that the sampling of spur

TABLE V.

Mean mineral content of middle leaves from different types of growth (1942).

Situation.	Month of sampling.	Type.	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)
B	July	Shoot	1·30	152	0·201	43·4	1·28
		Spur	1·94	112	0·201	49·4	0·92
	October	Shoot	1·75	185	0·251	55·2	0·92
		Spur	2·12	229	0·330	41·2	0·77
K	July	Shoot	1·20	82	0·201	47·4	1·48
		Spur	1·57	88	0·334	50·0	1·79
	October	Shoot	1·75	61	0·099	31·8	1·17
		Spur	1·94	212	0·169	47·8	1·82

leaves was to be preferred, since it enabled instances of potassium deficiency to be diagnosed more readily.

In those trees in which the basal leaves of long shoots and spurs were sampled in 1942, the mean values for the mineral content of the two leaf types do not differ significantly. For potassium, however, the interaction of leaf type with date of sampling almost reaches significance ($F=2{\cdot}909$, $n_1=2$, $n_2=20$) :

TABLE VI.

Mean potassium content (%) of basal leaves from different types of growth (1942).

Date of sampling.	Non-fruiting spur.	Long shoot.
July	1·87	1·48
August	1·66	2·04
October	1·74	1·91

A possible explanation is that in July the potassium in the middle leaves of the long shoot has provided a reserve to supply the needs of the younger leaves on the same shoot, and the resulting deficit is not made up till later in the season.

In 1943, only in the two trees B was more than one type of leaf sampled. The mean values for the three types of shoot (laminae only) are given in Table VII.

The only differences here which approached significance were those in the sodium content ($F=16{\cdot}917$, $n_1=2$, $n_2=2$). The other differences shown are in general agreement with the

TABLE VII.

Mean mineral content of middle leaves from different types of growth (1943).

	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)	Sodium (p.p.m.)
Non-fruiting spur ..	1·51	273	0·421	85·4	0·99	160
Secondary from fruiting spur	1·67	204	0·334	69·4	0·90	252
Long shoot	1·31	212	0·251	88·9	1·26	125

earlier figures, and more can hardly be expected from a batch of twelve samples only. In respect of calcium, the two types of spur have reversed their position, and the differences are very much smaller than in the 1940 samples; in respect of manganese, the long-shoot figure is anomalously high, this type of sample having contained least manganese in 1940. In respect of iron, magnesium and potassium the agreement is quite satisfactory.

Mineral composition of petioles.

As already stated, in the 1943 samples the petioles with midribs from each sample were analysed separately from the laminae, and the results show some striking differences. The data are given in Table VIII (more severely scorched leaves omitted).

The data were analysed statistically, using a logarithmic transformation as in all analyses described in this paper. The differences between petiole and laminae were found to be highly significant for all elements except potassium, the manganese content in the petiole being lower, that of calcium, iron, magnesium and sodium higher than in the lamina. The differences in sodium content are particularly noteworthy; in view of the general high solubility of sodium compounds in water, the localization of this element in the petiole cannot easily be explained on a purely physical or chemical basis; differential permeability phenomena are presumably involved.

The nature of the data demanded treatment as a split-plot design, and it was found with interest and some surprise that the error term between leaf parts was significantly greater than that between leaves in the data for iron and manganese ($F=4\cdot347$ and $3\cdot517$ respectively, $n_1=20$, $n_2=10$). This suggests that there are uncontrolled factors which operate simultaneously to increase the iron content of the lamina and reduce that of the petiole in an individual leaf, or *vice versa*—and similarly with manganese. This may be connected with the observation sometimes made (Rogers and Shive, 1932; Maier, 1942) that when a plant suffers from iron deficiency, iron may be deposited in an insoluble form in the conducting tissues.

TABLE VIII.

Mean mineral content of laminae and petioles of scorched and healthy leaves.

Situation.	Condition of leaves.	Part of leaves.	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)	Sodium (p.p.m.)
B, C ..	Healthy	Laminae	1·38	140	0·270	63·0	0·85	164
		Petioles	1·98	369	0·360	48·6	0·91	825
	Scorched	Laminae	1·39	205	0·248	64·1	0·83	187
		Petioles	2·10	399	0·390	48·6	1·01	811
E, G, I, L	Healthy	Laminae	1·29	207	0·108	65·9	2·20	166
		Petioles	2·14	334	0·165	58·8	2·24	797
	Scorched	Laminae	1·34	191	0·078	63·3	2·15	141
		Petioles	2·09	281	0·156	52·1	1·91	711

None of the interactions between part of leaf and the other factors is significant. There is a suggestion of an interaction between leaf part and deficiency in the potassium data ($F=2\cdot640$, $n_1=1$, $n_2=20$) which, for what it is worth, indicates that laminae are to be preferred to petioles for diagnostic purposes—in contradistinction to the results obtained by Ulrich (1943) in the grape-vine. This interaction also approaches significance in the iron data ($F=2\cdot575$, $n_1=1$, $n_2=20$).

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SUMMARY.

On three occasions during 1942, samples of scorched and apparently healthy leaves, otherwise fully comparable, were collected from the same twenty-four' apple trees. On one further occasion in 1943, samples were taken from twelve of the same trees. The laminae were analysed spectrographically for calcium, iron, magnesium, manganese and potassium ; for the samples collected in 1943, the petioles with midribs were also analysed separately, and data for sodium content were also obtained.

In eleven out of the twelve pairs of trees sampled, the scorch symptoms could be related to a low concentration of either magnesium or potassium in the foliage. A comparison of 21-year-old trees of Egremont Russet with trees of the same variety recently topworked on to Bascombe's Mystery showed that the foliage of the former contained more magnesium but much less potassium than that of the latter ; this suggests that the trunk can supply much more potassium than magnesium to the young leaves relatively to their requirements of the two elements.

The concentration of mineral elements in slightly scorched leaves did not differ significantly from that in healthy leaves, with the exception of magnesium, which was consistently lower in scorched leaves of trees deficient in magnesium. In severely scorched leaves, the manganese content was also lower than that found in healthy leaves on the same tree.

The only significant seasonal changes in mineral concentration were in calcium, the concentration of which in the leaf increased between July and October, and in magnesium, the concentration of which decreased in trees suffering from magnesium deficiency, but increased in others.

Observations on different morphological types of leaf in general confirmed the author's earlier results.

The concentration of calcium, iron, magnesium and particularly sodium was higher in the petiole and midrib than in the lamina ; that of manganese was lower. A high content of iron or manganese in the petiole, due to uncontrolled factors, was associated with a low content in the lamina, and vice versa.

In relation to the question of deficiency diagnosis by plant analysis, the following are the conclusions reached :

Severely scorched leaves should be avoided ; but slight scorch is no disadvantage, and may even facilitate the diagnosis of magnesium deficiency.

Sampling for the diagnosis of magnesium deficiency is best carried out fairly late in the season.

The advantage of spur-leaf over shoot-leaf samples for the diagnosis of potassium deficiency is confirmed.

There is some indication that lamina samples are preferable to petioles for diagnosis of potassium deficiency.

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STUDIES IN THE DIAGNOSIS OF MINERAL DEFICIENCY
III. THE MINERAL COMPOSITION OF DIFFERENT TYPES OF LEAF ON APPLE TREES IN EARLY SUMMER

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In an earlier paper in this series (Goodall, 1943), the results were reported of chemical analyses of leaves of different morphological types collected in September 1940 from trees in a manurial trial of dessert apples. Considerable differences in composition were demonstrated between the types of leaf analysed, and it was concluded that the basal leaf of the non-fruiting spur was the most appropriate type to sample in order to show nutritional differences in respect of potassium and manganese.

It was evident that these results would not necessarily apply at other times of the year; indeed, at other times the range of leaf types from which a sample could be taken would be quite different. Accordingly, in the early summer of 1941 a further set of leaf samples was taken from trees in the same manurial trial with a view to elucidating the relationships existing early in the growing season.

Experimental methods.

The plantation from which the samples were taken has been described by Hoblyn (1941). At the time of sampling, the analyses of the previous set of samples had not been completed; consequently the writer was unaware of the considerable differences in composition that existed between the trees at the east and west ends of the plantation, and sampling was unfortunately restricted to the easternmost block of trees. In this block eight plots that had received superphosphate (560 lb. per acre annually) were sampled, the differences in treatment studied thus being confined to those in respect of sulphate of ammonia (half the plots received 168 lb. per acre annually) and potash (from 1937 onwards, half the plots had received 224 lb. per acre of sulphate of potash every year, while the rest had received 448 lb.). Samples were taken from Cox's Orange Pippin trees only, two trees from each of the eight plots being included in this experiment, one on Malling rootstock XII (very vigorous) and the other on Malling rootstock IX (very dwarfing). On two of the plots, however, the trees on rootstock IX were growing so poorly that the samples required could not be obtained from them; accordingly samples were taken instead from trees receiving the same treatments in adjacent blocks.

The leaves were collected on May 14th, 1941, about a week before the trees were in full blossom. From the flowering spurs, basal, middle and apical leaves were sampled separately. From non-flowering shoots* the basal leaves only were sampled. Each sample consisted of two leaves from different shoots. Four samples of each type were collected from every tree.

The leaves were carefully wiped with muslin to remove any contamination, the midribs removed and rejected, and the laminae dried in a vacuum desiccator over silica gel. They were then ground by hand in an agate mortar and analysed in duplicate for the elements calcium, iron, magnesium, manganese and potassium by a spectrographic method similar to that used previously (Goodall, 1943); a detailed description of the method is now being prepared for publication. Since the leaves were small, the material available for a single analysis

* "Shoot" is used here as a general term. At this time of year, spurs and long shoots cannot be distinguished with certainty.

was sometimes little more than 1 mg.; consequently the analytical errors are rather high. All results are expressed on a dry-matter basis.

Experimental results.

1. *Differences between trees.*—In Table I is given the mean composition of all leaves gathered from each pair of duplicate trees.

None of the effects of soil treatment is significant. The potassium content of the leaves from trees receiving a double application of sulphate of potash is higher than that of the others; the difference approaches significance ($F=4\cdot336$, $n_1=1$, $n_2=4$), but is much less marked than in the samples collected in September 1940. The foliage of the trees receiving sulphate of ammonia contains a higher concentration of manganese than that of trees receiving none; this difference again approaches significance ($F=5\cdot046$, $n_1=1$, $n_2=4$; a logarithmic transformation was required before the data on manganese content could be analysed), and is similar to the difference found in the earlier data. An increase in the manganese content of plants following treatment with ammonium sulphate has fairly often been observed (Ruprecht and Morse, 1918; Snider, 1943).

Table I.

Mean composition of all leaves gathered from each pair of duplicate trees.

Rootstock.	Soil treatment.	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)
IX	PK	2·18	878	0·263	89	1·74
	NPK	1·80	757	0·235	125	2·04
	P2K	1·54	762	0·231	76	2·16
	NP2K	2·03	827	0·247	118	2·10
XII	PK	1·76	864	0·293	85	2·26
	NPK	1·46	975	0·287	126	2·30
	P2K	1·57	808	0·286	93	2·52
	NP2K	1·74	903	0·284	131	2·43

The foliage of trees on rootstock XII contains more magnesium and less calcium than that of trees on rootstock IX. These differences are significant ($F=20\cdot19$ and $16\cdot79$ respectively; $n_1=1$, $n_2=4$), and the difference in potassium content in favour of rootstock XII also approaches significance ($F=5\cdot543$, $n_1=1$, $n_2=4$). Vaidya (1938) also found the leaves of trees on rootstock IX to contain more calcium and less potassium and magnesium than on the other rootstocks he investigated.

2. *Differences between leaf types.*—In Table II is given the mean composition of each of the four types of leaf sampled.

Table II.

Mean mineral composition of different types of leaf.

Type of growth.	Leaf position.	Calcium (%)	Iron (p.p.m.)	Magnesium (%)	Manganese (p.p.m.)	Potassium (%)
Flowering spur	Basal	1·97	865	0·262	105	2·32
	Middle	1·58	888	0·252	105	2·26
	Apical	1·70	957	0·275	105	2·22
Non-flowering shoot	Basal	1·78	677	0·273	106	1·97

The differences among the types of leaf are very highly significant for iron and potassium ($F=9\cdot091$ and $8\cdot553$ respectively; $n_1=3$, $n_2=204$), the leaves from non-flowering shoots containing lower concentrations of both elements. The three types of leaves from flowering spurs are very much alike in mean composition, except for their calcium content which is higher in the basal leaves; this difference only just reaches significance ($F=2\cdot882$, $n_1=3$, $n_2=206$), and may perhaps be regarded as fortuitous.

The potassium contents show a marked interaction ($F=3\cdot802$, $n_1=3$, $n_2=204$) between leaf type and rootstock, as may be seen from the data in Table III. The difference in potassium content between non-flowering shoots and flowering spurs is substantial in trees on rootstock XII, negligible on rootstock IX.

TABLE III.

Effect of rootstock on potassium content (%) in different types of leaf.

Type of growth.	Leaf position.	Rootstock IX.	Rootstock XII.
Flowering spur	Basal	2·01	2·63
	Middle	2·07	2·45
	Apical	2·06	2·39
Non-flowering shoot	Basal	1·91	2·03

In iron content there is a significant interaction ($F=3\cdot798$, $n_1=3$, $n_2=204$) between leaf type and application of sulphate of ammonia, as is shown in Table IV. In flowering spurs of trees receiving sulphate of ammonia, the iron content is highest in the apical leaves; in the trees receiving none, the concentration in the basal leaves is highest.

TABLE IV.

Effect of sulphate of ammonia on iron content (p.p.m.) in different types of leaf.

Type of growth.	Leaf position.	Sulphate of ammonia applied annually.	
		None	168 lb./acre.
Flowering spur	Basal	947	783
	Middle	806	970
	Apical	880	1,034
Non-flowering shoot	Basal	678	675

The data do not indicate that the mineral content is affected by other interactions of the factors studied.

DISCUSSION.

As pointed out in the earlier paper (Goodall, 1943), the absolute values of the mineral composition data reported there should be treated with some reserve. Thus the two sets of data should be compared only very cautiously, even if account is taken of the fact that only four of the twenty-eight trees from which samples were taken were the same on both occasions, and that no leaf type is the same in both sets (the basal leaf on the non-flowering shoot, in the present paper, would include leaves corresponding with basal leaves from both V and L shoot types in the earlier paper). It is, however, worth drawing attention to the great difference in

iron content, which, in May 1941, was about ten times that found in September 1940. Further observations will show whether there is regularly so marked a change during the course of the season.

The differences between leaf types in the data now under consideration are very much smaller than those found in the samples gathered in the previous September. This is perhaps not very surprising ; the total mass of foliage in May is relatively small, and the reserves of nutrients available in the woody parts of the trees have, no doubt, been ample for all the leaves formed by this time. Also, all the leaves sampled had been developing almost simultaneously whereas, it will be remembered, the leaf samples of different types collected in September 1940 appeared in their composition to follow a sequence according to the order in which they had developed. Although the leaves of the flowering spur are initiated in succession, they all expand simultaneously as soon as the buds burst.

The fact that the difference in potassium content resulting from the application of sulphate of potash was less marked in May 1941 than in September 1940 has already been pointed out. This, again, is probably ascribable to the small mass of foliage at blossoming time relatively to the reserves of nutrients accumulated in the woody parts.

The small nutritional differences existing between trees in the early summer make it difficult to draw conclusions regarding the relative value of different types of leaf for diagnosis of nutrient deficiencies. Differences in potassium content between the two rootstocks are more marked in the leaves of the flowering spurs than in those of the non-flowering shoots. This, however, may be a specific rootstock effect on the distribution of potassium among different shoot types, and it would be unwise to accept it as evidence on the point at issue. It may therefore be concluded that at blossom time there is no indication that one type of leaf is preferable to another for diagnostic purposes. Nevertheless, it should again be emphasized that samples for comparison should be of the same morphological type—a point which might be of some importance if a nutritional disorder affected the amount of blossom on the tree.

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SUMMARY.

Leaf samples collected just before blossoming time in 1941 from Cox's Orange Pippin trees growing in plots receiving different dressings of sulphate of potash and sulphate of ammonia were analysed spectrographically for calcium, iron, magnesium, manganese and potassium. Two different rootstocks were involved. The basal, middle and apical leaves of flowering spurs and the basal leaves of non-flowering shoots were gathered and analysed separately.

On the vigorous rootstock (M. XII) the leaves contained less calcium, more magnesium and perhaps more potassium than on the dwarfing rootstock (M. IX).

The iron and potassium content of leaves from flowering spurs was greater than that of leaves from non-flowering shoots. The difference in potassium content was marked only in the trees on rootstock XII. In the flowering spurs, the iron content was greatest in the apical leaves for trees receiving sulphate of ammonia, but in the basal leaves for trees receiving none.

Differences in composition among leaf types and between soil treatments were less marked than in samples collected from the same source in the preceding autumn. This is ascribed to the smaller mass of the early summer foliage relatively to the reserves of nutrient elements in the woody parts of the tree.

The data provide no evidence for choosing one type of leaf rather than another for diagnostic purposes ; but they show once again the importance of basing comparisons between trees only on leaves of the same morphological type.

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FURTHER WORK ON PLANT INJECTION FOR DIAGNOSTIC AND CURATIVE PURPOSES

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This communication describes improvements made in plant injection methods since they were described in detail (Roach, 1938, 1939) and includes experience on a wider range of plants, both in this country and in South Africa. A brief note on the results obtained has already been published (Roach, 1940). The methods elaborated are now in use in many parts of the world, but the present is not a suitable time for making a comprehensive survey of the recent work of other investigators ; this is deferred until after the war. The present account is written as an addendum to the earlier papers, to which page references are given.

I. INJECTION FOR DIAGNOSTIC PURPOSES.

Concentration of solutions.—Responses have been obtained on a number of different kinds of plants with the following solutions, but it may be necessary to modify them, e.g. when work is done on new kinds of plants.

N	1%	Urea.
P	0·5%	Sodium dihydrogen phosphate.
K	1%	Potassium chloride.
Ca	1%	Calcium chloride.
Mg	0·5%	Magnesium sulphate.
Fe	0·025%	Ferrous sulphate+0·025% (by volume) sulphuric acid.
Mn	0·025%	Manganese sulphate+0·025% (by volume) sulphuric acid.
Zn	0·025%	Zinc sulphate+0·025% (by volume) sulphuric acid.
Cu	0·025%	Copper sulphate+0·025% (by volume) sulphuric acid.
Ni	0·025%	Nickel sulphate+0·025% (by volume) sulphuric acid.
B	0·1%	Boric acid.

High grade chemical reagents are quite satisfactory. The sulphuric acid is added to the metallic trace element solutions to prevent all risk of precipitation by hydrolysis. This has been a serious inconvenience in respect to the ferrous sulphate solution.

A. Interveinal leaf injection (cf. Roach, 1938, p. 18 ; 1939, p. 168).

Further experience with this method has confirmed the conclusion that a response may be detected more rapidly by it than by any other method. In an experiment by B. N. Lal (1944) at Malling a response to iron was observed in the Soya bean two days after injection. The method as originally described has, however, two serious drawbacks : it calls for delicacy of manipulation and it cannot be used in windy weather.

The apparatus described below (Fig. 1) has made manipulation easier, has removed the tendency for the contrivance to fall before the absorption is complete, and has increased its resistance to wind. The improvements consist mainly in the replacement of the long, relatively heavy, glass tube by a short, light one, made of cellophane, and the use (in one method) of a chisel-ended scalpel to make an incision of exactly the right width, the lips of which consequently clip the filter paper wick and hold it firmly.

Construction of the appliance.—The cups for holding the liquids to be injected are made from cellophane drinking straws (those without a decorative strip of tinsel). These are obtainable in several distinctive colours, which may if desirable be increased by marks in waterproof

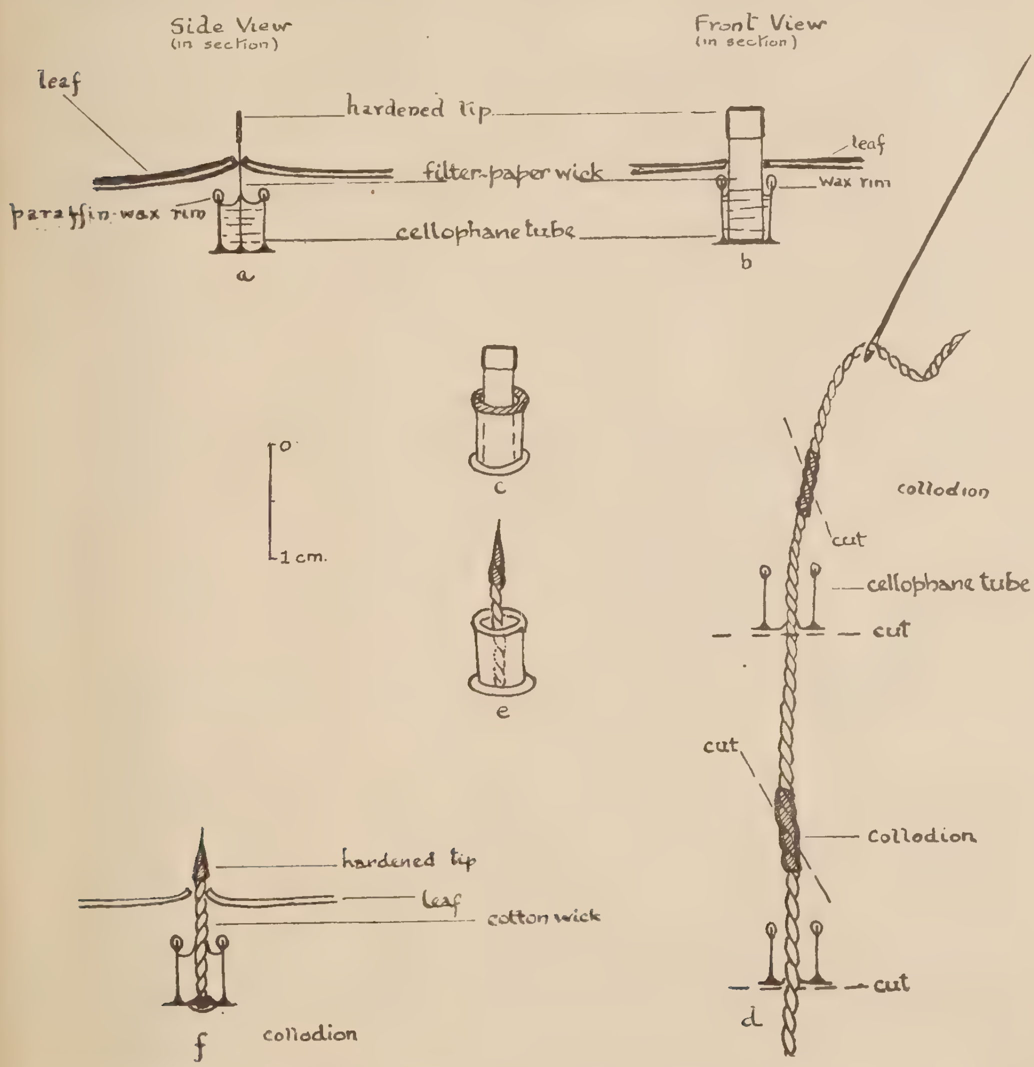

FIG. 1.

Apparatus for interveinal injection. Side and front sectional views of one form in use are seen in (*a*) and (*b*). It is drawn in the solid in (*c*). The manufacture of the other form is illustrated in (*d*). It is seen in the solid in (*e*), and (*f*) is a sectional view of it in use.

ink. If each colour or colour combination is used for a particular solution the little cups may be used a number of times without cleaning.

The straws are cut to the required length with the apparatus illustrated in Fig. 2. The straws are slid along the guide A, through the hole B in the vertical piece of box-wood C up against the stop D, which can be adjusted to give different lengths of straw, the length being

read on the protruding end E of the stop. A new safety-razor blade is mounted inside the wooden slide F, the component parts of which are shown in *b*, *c* and *d*. These components are fitted together so that the bottom edge of the middle part, shown in *c*, occupies the position shown by the dotted line in *d*. The safety-razor blade is held in a sloping position by two dowel pins G and H protruding from the side-piece shown in *d*, passing through the holes in the blade and through similarly lettered holes in the middle *c* and the other side-piece *b*. The three pieces of wood are held together by three nuts and bolts, J, K, L. When in use, the blade is thus held tightly against the side of C. When the slide is moved towards the straw along C the blade passes across hole B, cleanly cutting off the protruding length of straw without denting it, and it is caught in a suitably placed shallow box.

To lessen the tendency of the liquid to be drawn out by capillarity along the leaf surface when the underside is hairy, the rim of the cup is protected by a thin layer of paraffin wax (Fig. 1, a & b). The layer is applied by heating a little of the wax on a horizontal sheet of glass over a steam bath and dipping one end of each piece of tube into the thin layer of melted wax.

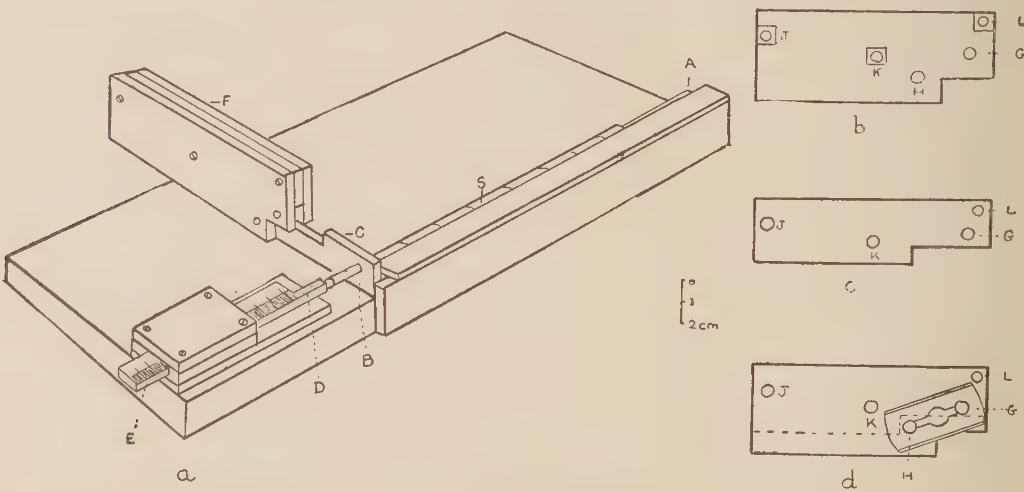

Fig. 2.

(*a*) Guillotine for cutting cellophane drinking straws into lengths suitable for injection cups. (*b*), (*c*), (*d*), details of F. For detailed description see text.

Discs to form the bottoms of the cups are cut from sheet cellophane with a leather-cutting punch of slightly greater diameter than that of the tube, a piece of card being placed between the cellophane and the brass block against which the cutter is pressed. Into the non-waxed end of each bit of cellophane tube is squeezed a drop of collodion* and the tube is then placed over one of the discs so that the collodion may seal the two together, thus closing the end of the piece of tube and forming a little cup. While the collodion is still liquid a filter paper wick is placed in the cup. When the cup is dry the tip of the wick is hardened by brushing it with a little dilute collodion. This soaks in and hardens about 2 mm. of the wick. Instead of a filter paper wick a cotton one may be used. For that purpose the cups are allowed to dry, ordinary unmercerized mending cotton is drawn up through the bottom of the cup with a very fine darning needle (Fig. 1, *d*, *e*, *f*). The cotton is cut off just below the bottom of the cup and a little longer than is necessary above. Collodion is squeezed over the bottom of the cup to secure the thread and seal the hole ; it is also smeared over the protruding end of the thread, leaving about 2-3 mm. untreated above the rim of the tube. When quite dry, the hardened end is

* An adhesive sold in convenient tubes and known as Durofix, is suitable for this purpose.

sharpened to a point by cutting slantwise with a razor blade, as shown in Fig. 1 (*d*), which also shows how several of such tubes may be provided with cotton wicks at one and the same time.

Both types of cup can be filled with the required liquids by using an ordinary dropping bottle with a slender delivery tube.

The scalpels (Fig. 3, A and B) are made by breaking a suitable piece from a thin safety-razor blade of the Gillette type held in parallel-jawed pliers and cementing it between two flat wooden spills with collodion. A number may be clamped between boards to dry overnight, after which collodion is run around the joint between the blade and the wood to strengthen it.

For work in the field it has been found convenient to carry the various items of the apparatus in a box fitted so that each dropping bottle occupies its own compartment in a row and has opposite it two compartments, one for each type of its distinctively coloured injection cups.

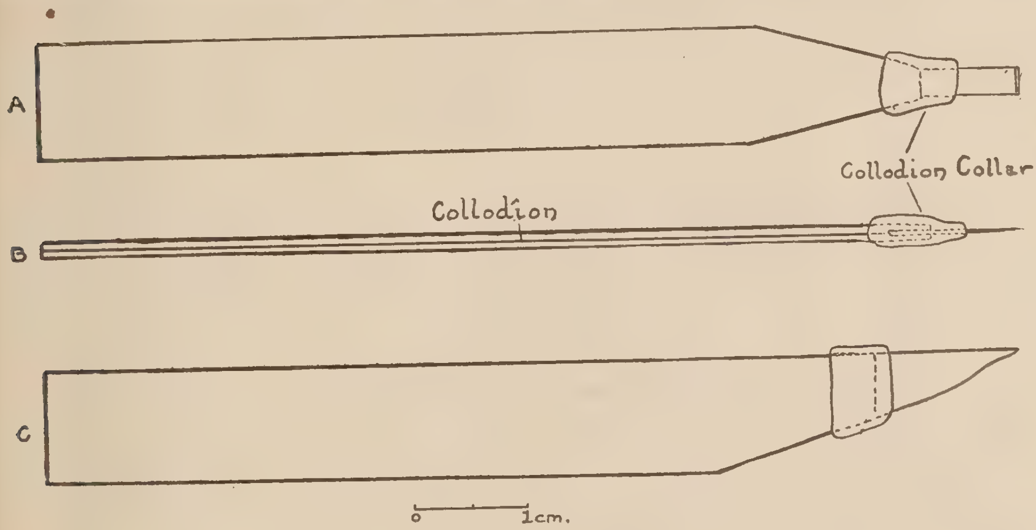

FIG. 3.

Scalpels suitable for diagnostic injection. A, front, and B, side view of chisel-end scalpel for interveinal injection. C, front view of scalpel for leaf-stalk injection.

B. LEAF-STALK INJECTION (cf. Roach, 1938, p. 27; 1939, p. 180).

This is by far the most generally useful of all diagnostic injection methods. It is at present in routine use for testing the effect of essential elements singly and in combination, and many thousands of injections of this type have been done. Experience has proved the desirability of carrying out the test with each solution at least in quadruplicate. Usually about a dozen elements are involved, which entail about fifty injections. Water injections should also be included.

Construction of apparatus.—The apparatus found most convenient is shown in Fig. 4, A and B, and two methods of using it are illustrated in Fig. 5, A and B. Apparatus shown in Fig. 4, B is for use with smaller leaves.

For lightness, tubes are blown from the thinnest walled quill glass tubing. These are obtainable from Messrs. C. L. Muller, 6 Parton Street, Red Lion Square, London, W.C.1.

A suitable length of lead wire is held against the tube in a spring clothes peg. Collodion is applied to the bottom of the lead wire so as to stick it to the tube; a little more is applied to the wire near the open end of the tube. While the collodion is still liquid two turns are made round both ends of the tube with thread, which is then covered completely with a layer of

collodion. When dry the clothes peg is removed and collodion is applied along the whole length of lead wire in contact with the tube. In this way the exterior of the whole contrivance is left quite smooth and without projections which might scratch the leaves in a wind. Finally, a thin band of melted paraffin wax is painted round the inner rim of the tube. This tends to prevent the liquid from running out of the tube, even when it slopes mouth downwards.

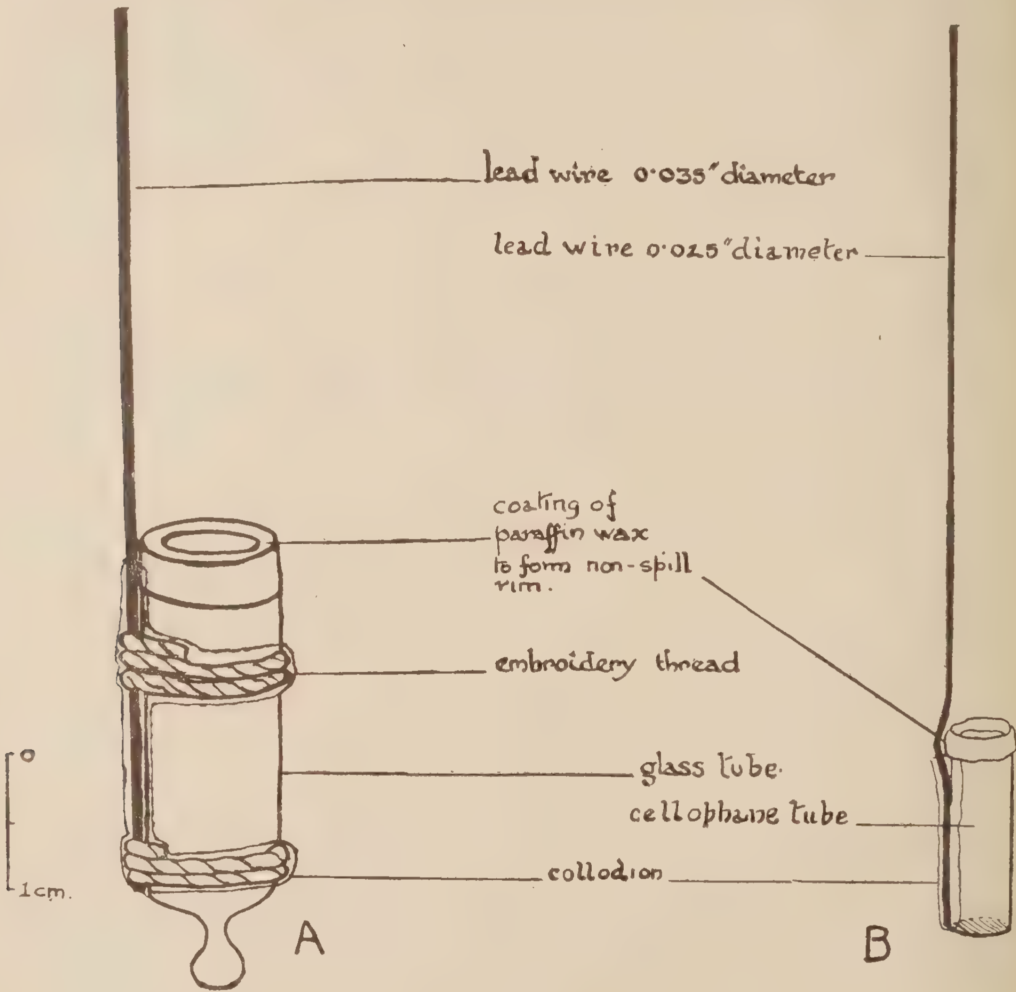

FIG. 4.
Cups for leaf-stalk injection. A for larger and B for smaller leaves.

For slender leaf-stalks the single coil of the wire shown in Fig. 5, A is sufficient to bend the stalk down. Stiff leaf-stalks can be bent down by using a piece of ·025 in. diameter lead wire attached to the basal knob of the tube and with its other end coiled around the stem of the plant (Fig. 5, B). Both for maximum speed in working and for minimum damage to the plant the wire should be twisted as little as possible.

The type of scalpel found most convenient for leaf-stalk injections is shown in Fig. 3, C.

It is easily held in the mouth, or even stuck to the moistened underlip, between operations, and its lightness is a distinct advantage in use.

As already pointed out (Roach, 1938, 1939), the reliability of a diagnosis made by the leaf-stalk injection method depends on how closely the distribution of the improvement (if any) in leaf colour (and sometimes the growth increase) corresponds with expectation based on experiments with dyes, and on the degree of likelihood of a similar distribution of colour variation occurring naturally. Hence before starting such injections on a fresh type of plant it is necessary to do a sufficient number of experiments with dyes and to make a survey of the naturally occurring

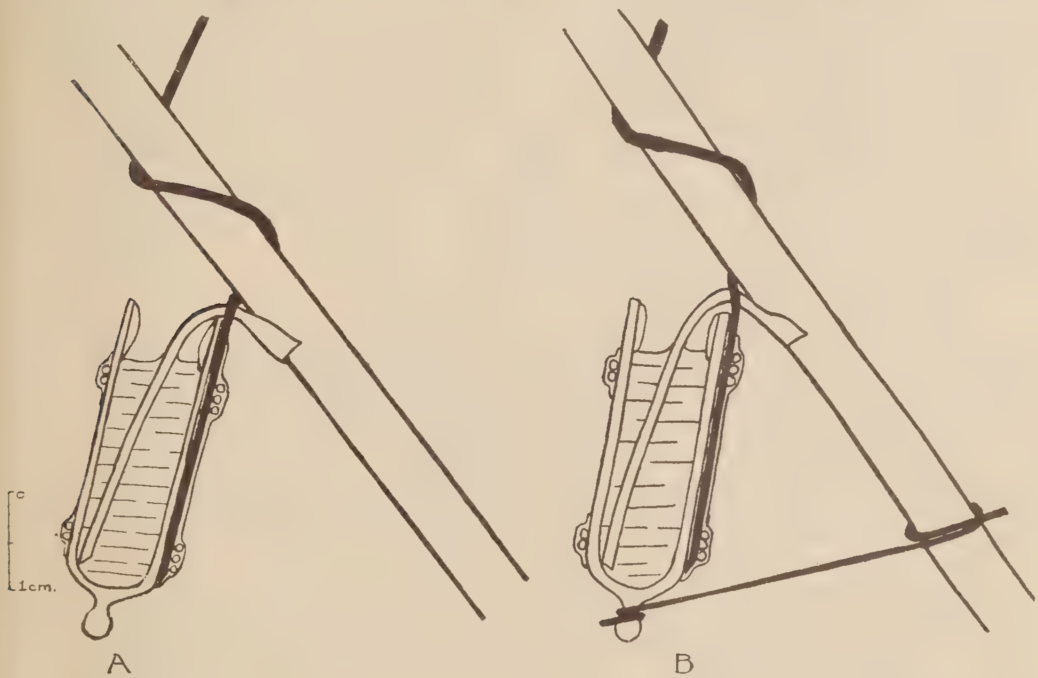

Fig. 5.

Method of attaching injection cups to the plant stem. The method shown in A is used unless the leaf-stalk is too stiff, when the method seen in B is used.

variations in colour and size which might be mistaken for the result of the experiment ; shoots with leaves showing such variations must, of course, be avoided.

In such plants as the apple, the distribution of injected liquids may be predicted within narrow limits, and there are all stages between this type of material and the aubergine, in which the distribution is so variable as to make this type of injection useless for diagnosis. There are similar differences between species of plants in respect of the degree of variation in colour and size of the leaves. The degree of variation in the leaves of a given plant depends largely on the degree of uniformity of the soil.

C. Summary of experience with various plants.

Extensive injection work has been done for the most part on relatively few kinds of plants but enough has also been done on a number of other kinds to suggest the most hopeful methods to try on them. The following notes summarize our present knowledge. The plants are arranged alphabetically.

Apple.—Frequent references have been made to methods suitable for the apple (Roach, 1938, 1939). The leaf-stalk method is the most generally useful. The commonest type of distribution of injected substance has already been described (Roach, 1938, pp. 27-29; 1939, pp. 181-183). The only really commonly occurring divergence from this pattern is one in which the two leaves immediately above and below the injection point respectively do not become permeated. In a recent experiment with 1 per cent. acid fuchsin on the variety Lord Derby, in which 100 shoots were injected, the first leaf below the injection point in 10 shoots was free of dye, and in one shoot only were the leaf below and the leaf above this point free of dye.

Apricot.—The distribution resulting from the injection of a regular shoot of the apricot with dye solution by the leaf-stalk method is similar to that shown in Fig. 19, Roach, 1938 and text Fig. 17, Roach, 1939. Judging from the small number of shoots injected, it would seem that shoots in which the arrangement of the leaves is irregular is somewhat common. These must be avoided, because they give unpredictable injection patterns.

Aubergine.—Dye solution injected into the aubergine by the interveinal method moves freely, and even medium sized veins do not form definite barriers to permeation, but the method seemed worth trying. The plants used were unusually healthy, however, and no response was obtained to mineral injection. The distribution resulting from leaf-stalk injection was irregular and this method appeared useless for diagnosis.

Broad Bean.—See Hill and Roach, 1940, pp. 518-520.

Cape Gooseberry.—A few experiments carried out late in the season with vigorous Cape Gooseberry plants below deciduous fruit trees in the Western Cape Province, South Africa, suggest that this plant would lend itself to diagnosis by the leaf-stalk method, and that the first, second and third leaves above the injection point become permeated sufficiently unequally on their two sides to be useful for diagnosis. Strong colour responses and obvious growth increases were obtained. Shoots in which the arrangement of the leaves is not quite regular must be avoided. Although not of great commercial importance this plant is nevertheless worth testing as an indicator plant, especially for diagnosis when the fruit trees in the same plantation are not in growth.

Citrus.—A few experiments were carried out on orange trees between the seasons of growth flush. These suggested that the leaf-stalk method is well worth trying on trees making vigorous growth. An abnormal type of shoot common on trees between growth flushes must be avoided, because leaf-stalk injection of them results in a general distribution of the injected liquid throughout the whole shoot. These shoots are easily recognized because their leaves are small and become progressively smaller from the base of the current year's growth to the tip. The experiments done on normal shoots suggest that the distribution may be predicted with sufficient accuracy for diagnosis. The commonest type of distribution corresponds closely to that given by the pear (Roach, 1938, p. 33; 1939, p. 188). Citrus leaves often become bleached in sunlight and this must be borne in mind in making a diagnosis. No positive response was obtained in any of the few score injections of nutrients carried out during a visit to the Union of South Africa, but none of these was done under the best conditions for getting a response. Experiments under better conditions, i.e. when the trees are making vigorous growth, are therefore highly desirable.

Coffee.—See Roach, 1938, p. 35; 1939, p. 189.

Currant.—See Roach, 1938, p. 33; 1939, p. 188.

Gooseberry.—See Roach, 1938, p. 33.

Gousblom (Arctotis).—Work was done on this plant (Compositae) because it is a self-sown cover crop commonly found in some of the orchards of the Western Cape Province and it is in vigorous growth when the fruit trees are leafless. The pattern resulting from leaf-stalk injection resembles that of the apple.

Guava.—Experiments with dyes only have been done on this plant. Interveinal injection results in the permeation of the area without any dye crossing the bounding secondary veins. Leaf-stalk injection gives the same distribution as in coffee and the Hydrangea.

Hop.—See Roach, 1938, p. 35.

Japanese Plum.—The leaf-stalk method is the most useful one for this plant and it gives the same distribution as in the common plum. Care must be taken, however, not to use shoots developing from adventitious buds on old wood, because leaf-stalk injection on such shoots results in permeation of the leaves of practically the whole shoot ; no leaves became permeated on one side of the midrib and not on the other.

FIG. 6.

The distribution of liquid resulting from the injection of a lupin leaf through a cut leaflet tip.

Loquat.—Experiments with dyes only have been done on this plant. The extreme hairiness of the loquat leaf does not interfere with interveinal injection because the hairs are not easily wetted. Dye solution injected through an interveinal incision permeates the interveinal area thoroughly, and, in addition, enters a band near the midrib nearly reaching the leaf tip. Comparison must therefore be made mainly between one side of the leaf and the other and not between contiguous interveinal areas. The effect of a high concentration may be seen by comparing the interveinal area containing the incision with the one on the opposite side of the leaf, and the effect of a lower concentration by comparing most of the injected half leaf with the other half. Leaf-stalk injection brings about the permeation of leaves of the whole shoot and can therefore be used for experiments in which a unit of this size is desired.

Lupin.—The leaflet-tip method of injection has been found to be applicable to a number of types of lupin grown as cover crops in South Africa and in gardens in this country. A typical result of injecting dye is shown in Fig. 6. The effect of injected nutrients is usually seen in the

shaded and black areas. Leaf-stalk injection tends to result in the second leaf above, the first and third leaves below, becoming permeated on the proximal but not on the distal side. Limited experience with this method suggested that it is worth trying as well as the leaflet-tip method. The leaf-stalk one is the easier to carry out.

Peach.—The leaf-stalk method of injection is the most useful and gives the same pattern as in the apple.

Pear.—See Roach, 1938, p. 33; 1939, p. 188.

Plum.—The leaf-stalk method of injection is the most useful and gives the same pattern as in the pear.

Potato.—Experience in the field since the publication of the paper by Hill and Roach, 1940, has proved the most useful method to be one in which the stalk of a leaflet halfway along a leaf is immersed. This results in the leaflet above and the one below becoming permeated, more heavily on the proximal than on the distal side. Slight effects appear to have resulted occasionally from the injection of water into a few leaves by this method.

Raspberry.—See Roach, 1938, p. 30; 1939, p. 185.

Rose.—The leaf-stalk method is the most useful and gives a similar pattern to that in the apple.

Soya bean.—See Lal. The interveinal method is the most useful.

Strawberry.—See Roach, 1938, p. 26; 1939, p. 179.

Tobacco.—See Lal. Both the interveinal and the leaf-stalk methods are useful.

Tomato.—See Hill and Roach, 1940, pp. 506-512.

II. INJECTION FOR CURATIVE PURPOSES.

A. Injection of liquids (cf. Roach, 1938, p. 52-60).

The method illustrated in Fig. 7 has been found satisfactory for injecting large trees. A hole is bored through the bark just down to the wood with a sharp $\frac{3}{8}$-inch Irwin bit. The hole is continued into the wood with a sharp $\frac{1}{4}$-inch similar bit. Into this hole is pushed a cylindrical

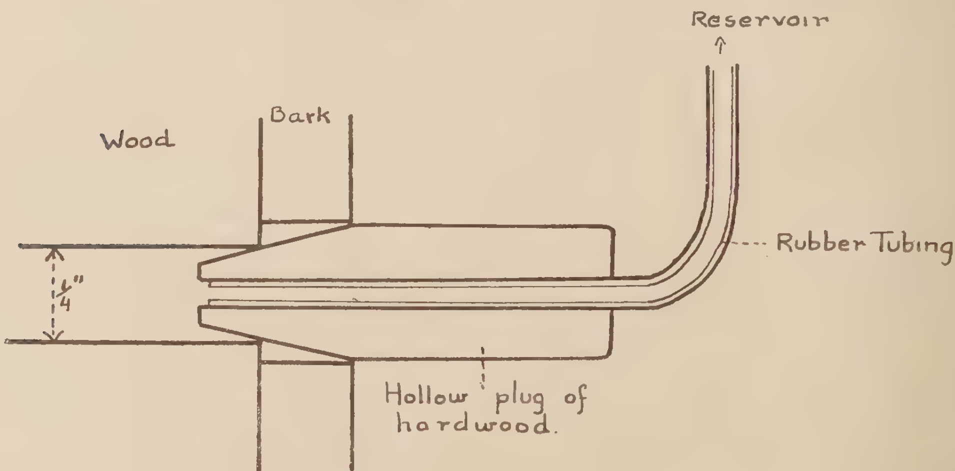

Fig. 7.
Method for leading liquid into the injection hole in the tree-trunk or branch to be injected.

plug of hardwood $\frac{3}{8}$-inch diameter, tapered at one end to a little less than $\frac{1}{4}$ inch and bored to take the narrow-bore rubber tubing (such as is used for bicycle tyre valves) leading to the reservoir. It is important that the boring be done by a genuine Irwin bit, kept clean and razor sharp, otherwise the cut edges will be ragged and a good joint with the plug will be impossible, nor will the hole heal rapidly and cleanly. The degree of plugging of the cells in the wood next to the bark is not found to slow up absorption appreciably except when there is only a thin ring of living wood, e.g. in a plum tree affected by Silver Leaf disease, when the method described earlier should be used (Roach, 1938, p. 53).

B. Injection of solids (cf. Roach, 1938, p. 68; 1939, p. 217).

The advantages of injecting a solid instead of a liquid are that little apparatus is necessary, the handling of volumes of liquid comparable with those required for spraying is avoided, and the process is completed in one operation. The use of the solid method became essential when it was found that cherry trees commonly absorbed little or no liquid (Duggan, 1943). The most serious drawback to the solid method is the amount of mechanical damage done because of the necessity of having so many holes in the trunk. Thus, about ten times as much wood is removed in making the holes for solid, as is necessary for liquid injection. Bennett's (1931) recommendation amounts to a hole of $\frac{1}{2}$-inch diameter every 3 inches of circumference of trunk or branch.

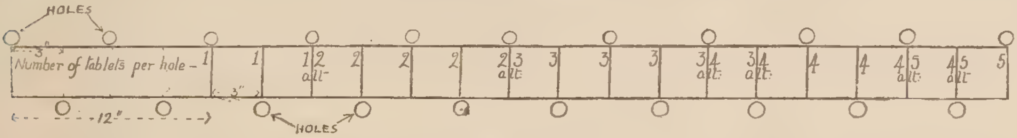

Fig. 8.

Calibrated rubber band for spacing holes for injecting a tree with substances in tablet form. The band is wrapped round the tree and the line nearest where the beginning overlaps the other end gives the number of tablets to be inserted into each hole.

This exposes the wood to risk of invasion by disease-producing organisms, and it must be reduced to a minimum. It has been found that any reduction in the number of holes results in incomplete distribution of the substance throughout the tree (Duggan, 1943).

The aim of the method now to be described is to encourage rapid and clean healing of the holes. The essentials are a clean cut and the prevention of contact between the introduced solid substance and the bark. The spacing of the holes around the trunk is most conveniently done by wrapping round it a rubber band (such as shown in Fig. 8) on which a mark has been made every 3 inches, first securing the end of it with a pin and stretching the band slightly, if necessary, to make the first mark coincide with another one. Against this latter mark will be found the number of grm. tablets of substance to be introduced into each hole. To save using tablets of smaller size to get the exact dosage for each hole when the amount comes to an odd half gm. it is permissible to use the whole number above and below alternately. It is important to note that if branches have died back the amounts injected must be reduced.

A hole of $\frac{5}{8}$-inch diameter is bored through the bark just down to the wood and is continued into the wood with a $\frac{1}{2}$-inch bit using the same centre (Fig. 9, A). As before, it has been found essential to use Irwin bits kept clean and razor sharp. A cork of $\frac{1}{2}$-inch diameter at the smaller end and $\frac{1}{4}$ inch long is placed in the end of a metal tube of $\frac{1}{2}$-inch internal and $\frac{5}{8}$-inch external diameter, and externally bevelled at the other end. The solid is obtained in 1 grm. tablets (lighter ones for smaller trees) and the requisite number are placed in the tube (Fig. 9, B). The tube is now inserted into the hole in the bark until its bevelled mouth presses on the margin of

the hole in the wood (Fig. 9, C). The end of a plunger provided with a handle is now placed against the cork and pushed home, driving tablets and cork into the hole in the wood (Fig. 9, D).

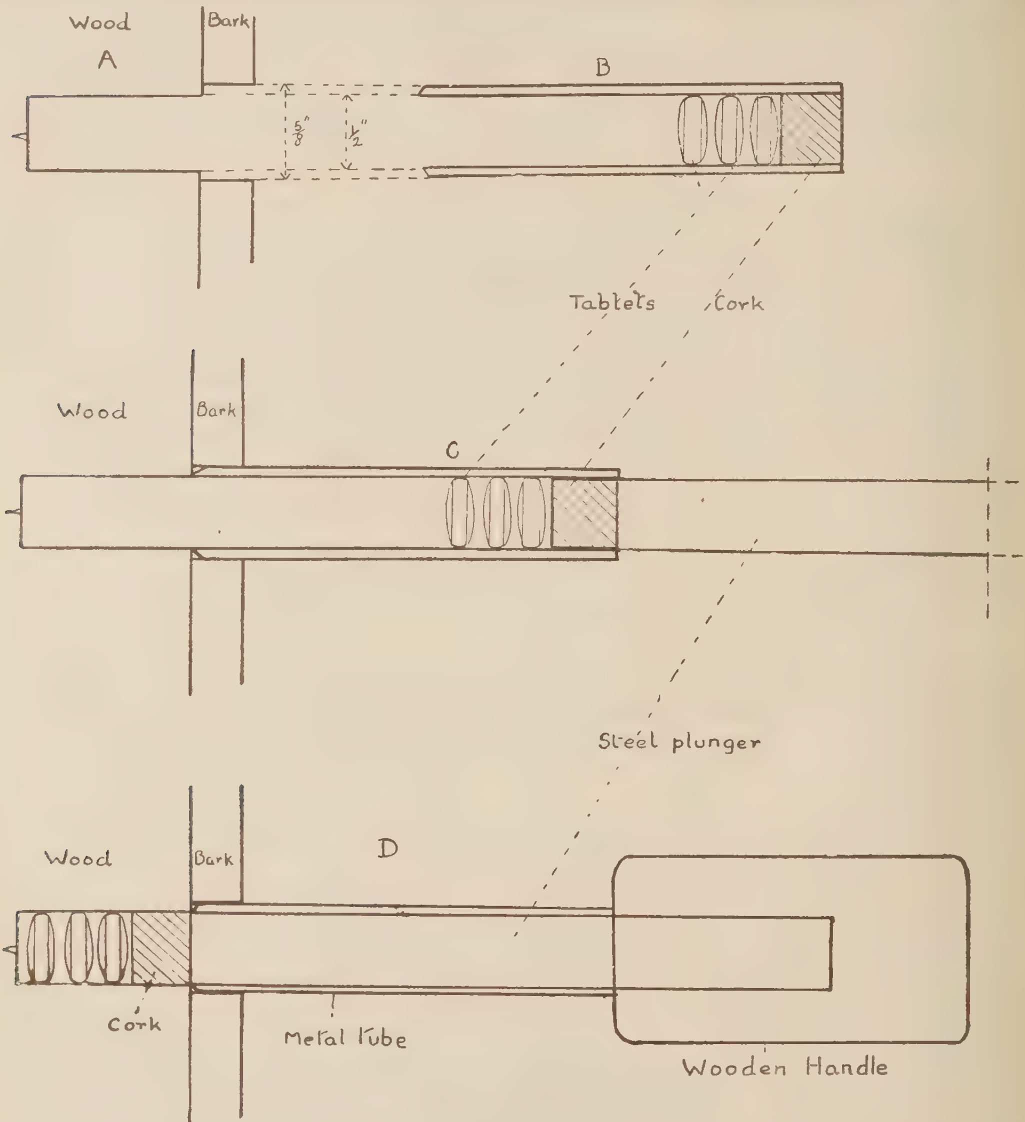

FIG. 9.

Sectional drawings illustrating method for injecting a tree with substances in tablet form. A, Shows holes bored through bark and wood. B, Metal tube with cork and tablets in position. C, Tube inserted in hole in bark against the mouth of the hole into the wood. End of plunger ready to push cork and tablets into hole in wood. D, Cork and tablets pushed home into hole in wood.

The length of the plunger protruding from the handle is exactly the same as the length of the tube measured along the inside wall. The outer end of the cork, therefore, lies flush with the surface of the wood and forms a platform over which healing takes place rapidly. Holes bored

in the winter or early spring heal during the succeeding summer and are barely visible by the autumn.*

Curative treatment has so far been done mainly on large trees. For small trees the size of the hole must be suitably reduced (see Bennett, 1931, p. 7, Table 3). For trees with soft bark the apparatus shown in Fig. 36 (Roach, 1938) makes the neatest holes.

SUMMARY.

Improvements in the appliances hitherto developed by one of the authors and his collaborators and used for plant injection with liquid for both diagnostic and therapeutic purposes and with solid materials for therapeutic purposes are described in detail and fully illustrated. They include the reduction in weight of the small containers employed for interveinal and leaf-stalk injection by the substitution of cellophane for glass. Special tools for making the cellophane cups are described and illustrated and the sources from which the requisite materials can be obtained are mentioned.

Brief notes are given on the experience gained both in this country and South Africa in the use of injection methods with twenty-five different kinds of plants, including fruit trees and bushes, some forage plants, hops, potatoes, tomatoes, tobacco and a few others. Paged references are given to information contained in previous papers on the subject.

In connection with injection for curative purposes, an improved method for the injection of solids into a tree is described and figured. It consists in drilling a small hole through the bark, continuing it at a slightly decreased diameter into the wood, introducing with the help of a metal tube and plunger the necessary chemicals in tablet form into the wood and sealing them there with a cork disc, over which the bark readily heals. By this method a minimum of mechanical damage is done to the tree.

ACKNOWLEDGMENTS.

Figs. 2, 3, 4, 5, 11 and 12 were kindly drawn by Miss M. E. Bunyard, to whom the writers are duly grateful.

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* Mr. C. R. Thompson, Assistant Advisory Officer in Commercial Fruit Growing to the Kent Education Committee, has used an apparatus differing slightly from the one just described. A description of this has appeared in the *Market Grower* for Oct. 14, 1943, pp. 12-13 and for Oct. 21, p. 15, since this paper went to press.

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COVER CROPS FOR FRUIT PLANTATIONS

I. SHORT TERM LEYS

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Introduction.

In the past large amounts of farmyard manure or other organic substances were applied each year to orchards, but as supplies of these materials became scarcer many have had to do without. In some orchards, under such conditions, marked deterioration of the soil structure has been observed ; and at the same time signs of unsatisfactory health conditions of the leaves have appeared, suggesting deficient or unbalanced nutrition. Various types of leaf scorch and excessive susceptibility to spray injury are among the symptoms which have increased as the application of organic manures has decreased. Conversely, renewal of the organic supplies has, in some cases, caused amelioration of certain of these troubles. The exact mechanism of some of these effects has yet to be determined, and it should be recognized that in certain cases cures have been affected by inorganic substances. Nevertheless, the addition of organic matter to the soil appears to provide a safe and convenient method of ensuring a balanced nutrient supply in a suitable form.

This paper describes experiments to test the suitability and value of various cover crops which can be grown under orchard trees, and afterwards either incorporated in the soil by discing or ploughing in, or cut and left to rot on the surface. This practice has the great advantage that the organic matter is produced where it is used, so saving handling costs. Naturally the cover crop must occupy the ground at the same time as the fruit trees, hence great care may be necessary to avoid unfavourable reactions between tree and cover crop. Besides being of immediate practical importance, the problem involves an interesting ecological study of the relation of one crop to another, and of the deliberate mixing of crops to avoid some of the failings of a monoculture.

In practice a cover crop is usually a bulky green one, and if ploughed in the practice is known as green manuring. Grass and grass mixtures are types of cover crop, and their growth in orchards in America is usually called " sod culture ". The experiments in progress at East Malling fall into three main categories. (A) Short term leys, (B) Long term leys, and (C) Annual cover crops. The first of these will be described in detail in this paper, following a review of the literature ; and later papers will describe the other two.

Review of literature.

The literature on cover cropping and green manuring is extensive. The Imperial Bureau of Soil Science (Anon., 1931) published an excellently indexed bibliography and review of the subject, containing 437 references. Pieters (1927) in his book " Green Manuring " has 352 references, and Waksman (1930) in his book " Humus " 1,311 references. Most of this work refers to arable crops, though some of it deals with citrus and tropical fruits, and a little with temperate orchards.

There is little published research work on cover cropping under fruit in Britain, for while grass orcharding is an old and common practice with standard trees, especially in the West (Hoare, 1928), much modern top fruit culture is with bush trees under clean cultivation ; and the use of temporary cover crops had been but little tried.

The Duke of Bedford and Spencer Pickering (1919) noted, at Woburn and elsewhere, that grassing down fruit trees greatly checked tree growth. Their conclusion that this was due not

to competition for water and nutrients but to a toxic effect has not been supported by later work, however.

Turner (1935) published a brief report of tests of various swards under dwarf pyramid apple trees at Cannington, Somerset, and showed that while some grasses checked tree growth very severely, a suitable grass and clover mixture gave good results, and improved the fruit quality. Hoblyn and Bane (1934) reported on the effect of grassing down two bush apple plantations at East Malling, again showing a checking effect on tree growth and an improvement in fruit colour. They stressed the importance of a correct adjustment of the nitrogen supply to the sward and the tree. Wallace (1929) reported cases of the cure of chlorosis in apple leaves by grassing down, and also (1928) cases where grassing down reduced symptoms of leaf scorch, a disorder which he showed to be associated with potash deficiency. He considered that grassing down alone was not a generally satisfactory method of curing this type of leaf scorch, however, and that the benefit was obtained mainly by lowering the nitrogen supply and so decreasing the N/K ratio. Some practical notes on the culture of various possible cover crops for British orchards, including preliminary details of the present trials, were published by Rogers (1941).

In Germany, Schulz (1936) considered the reciprocal influence of under-crops and tree fruits, and noted that water supply may be a limiting factor. Meijers (1939) in Holland, and Berkner *et al.* (1939) in Germany concluded that leguminous cover crops were much more satisfactory than non-legumes.

In the U.S.A., cover cropping has played an important part in orchard management, and some valuable information on its effects there is available. Collison and Harlan (1933) showed the good results arising from grass, lucerne and red clover swards in an orchard of good vigour in New York State. Collison (1933) and Collison and Carleton (1942) showed that continuous clean cultivation resulted in depletion of the organic matter and nitrogen in the soil and led to risk of erosion ; cover crops, however, including both legumes and grasses, and annual and perennial crops, maintained or increased soil organic matter and nitrogen, and lessened erosion. Perennial swards were most effective. The effects on the trees themselves were not tested in these trials. Shaules and Merkle (1939), in a survey of soil organic matter and nitrogen under various cultural conditions in Pennsylvania orchard localities, concluded that clean cultivation was soil-depleting, but that leguminous cover crops could satisfactorily maintain an orchard soil. They considered sod culture superior to either clean cultivation or the use of annual cover crops under their conditions.

Collison, Beattie and Harlan (1933) found, from lysimeter experiments, that a lucerne crop fixed nitrogen from the air equivalent to 260 lbs. per acre per year. Murneek (1936) also emphasized the significance of annual leguminous cover crops in supplying orchard soils with nitrogen. He estimated that a heavy crop of hairy vetch added nitrogen to the soil equivalent to the amount contained in a dressing of 15 to 20 tons of stable manure. The importance of nitrogen in maintaining the growth and production of trees under grass was further stressed by Collison (1940) in a paper discussing various soil management methods. Partridge and Toenjes (1937) pointed out the danger of soil erosion in sloping orchards, and the value of grass as a control measure.

With regard to tree growth and cropping, Stokes *et al.* (1932) reported a substantial increase of growth of pineapple oranges in Florida, as measured by trunk cross-section, after turning-in annual cover crops over a period of six years. McCue (1918), in seven years' experiments with cover crops in peach orchards in Delaware, concluded that cover cropping had increased the fruit yields. West and Howard (1938), in a good trial with oranges under irrigation in New South Wales, found that a winter green manure crop increased both growth and yield, over the clean cultivated control ; lucerne, however, decreased growth and yield, owing to strong competition for water.

E. F. Palmer and Van Haarlem (1944), in a review of orchard soil management practices, showed that, in a 15-year experiment at Vineland, grass sod, though checking the tree in

earlier years, resulted in as high a total yield over the period as that from clean cultivation throughout.

R. C. Palmer, *et al.* (1941) compared single plots having (i) continuous cover cropping with hairy vetch, (ii) clean cultivation + 10 tons farmyard manure per acre per annum, and (iii) 600 lbs. per acre per annum of a 4-8-12 artificial manure. Of these plots (ii) resulted in the highest yield of fruit but it also contained the most added nutrients.

Several workers have found a connection between organic matter and availability of various nutrients. Jensen (1917), after a series of analyses of Citrus soils, concluded that the addition of green manures to the soil increased the availability of phosphoric acid, calcium and magnesium. Clawson (1916) and Morris (1923) noted that apple rosette was cured by three years of cover cropping, and Skinner and Demaree (1926) showed that pecan rosette was cured by ploughing in green manure crops or heavy dressings of organic manures. Since then Ward (1939) and others have shown that these troubles are associated with lack of zinc. Wallace's (1928, 1929) results on the cure of marginal leaf scorch and chlorosis by grassing down have been mentioned above. Thus strong evidence is at hand to show that some cover crops can make available for the tree elements present in the soil in form or amount otherwise of no avail to it. This evidence is strengthened by the trials described below.

Material and methods used.

In this experiment the effects of five cover crops (i) ryegrass (*Lolium perenne* and *L. italicum*), (ii) red clover (*Trifolium pratense*), (iii) a clover and ryegrass mixture, (iv) lucerne (alfalfa) (*Medicago sativa*), and (v) natural weeds (mainly *Poa*), were compared with that of clean cultivation. The experiment occupied a 2-acre plantation of bush apple trees on the dwarfing rootstock No. IX, spaced 12 ft. apart, and 12 years old when the crops were sown.

The five cover crops were replicated five times, in a Latin square, each sub-plot containing four Cox's Orange Pippin trees, four Beauty of Bath, one Worcester Pearmain, and one Duchess' Favourite, with guard trees all round. Each sub-plot had an area of 288 square yards. Two clean cultivated strips, each having five sub-plots as described, ran through the plantation, as shown in the plan, Fig. 1. This arrangement was adopted so that normal implements could be used. There were thus 50 experimental trees under each cover crop, and 100 under clean cultivation.

The soil on which the experiment was carried out is a medium sandy loam, usually from 3 to 5 ft. deep overlying Ragstone, a porous calcareous rock. Full mechanical analyses of it have already appeared (Rogers, 1939); chemical analyses are given on page 126.

Rainfall.—The average annual rainfall at East Malling for the past 26 years was 26·27″. During the four years 1940-3 the actual totals were 27·01″, 26·00″, 26·25″, and 25·70″ respectively, i.e. in no case deviating more than 0·74″ from the mean. The totals for the months May to September were more variable, being 6·24″, 10·78″, 11·61″, and 11·04″ in the years 1940-3 respectively, as against a 26-year average of 9·62″. Thus, during the experiment, the first summer was unusually dry, and the second, 1941, when the cover crops occupied the soil for the whole year, was slightly wetter than usual, due mainly to unusually heavy rains in August. In 1942 and 1943 also, after the cover crops had been turned in, the summer rainfall was slightly above the average.

Management.

The seed of the cover crops was broadcast in May, 1940, and harrowed in. All grew well, though the clover and lucerne plots became invaded later with a little grass. The natural vegetation which grew on the weed plots consisted (after a weak crop of "fat hen" (*Chenopodium album*) in the first year), mainly of weak-growing grasses, particularly *Poa annua*.

The orchard was subjected to the normal routine of tar oil, petroleum and lime-sulphur sprays for pest and disease control. No difficulty was experienced from spray damage in the cover crops, for they were all well established by the time the first winter spray was applied in 1940-41.

It was expected from the outset that nitrogen would be the main fertilizer requirement of the cover crops, but since the growth of the trees already indicated a fairly high nitrogen status, the fertilizer given was restricted to 168 lbs. per acre of sulphate of ammonia applied in July, 1941, and a similar amount in May, 1942, just before the cover crops were disced in. This was probably insufficient for the best results, as later observations showed. A further 224 lbs. per acre was given in March, 1943. The applications were made to the whole area, including the clean cultivated strips.

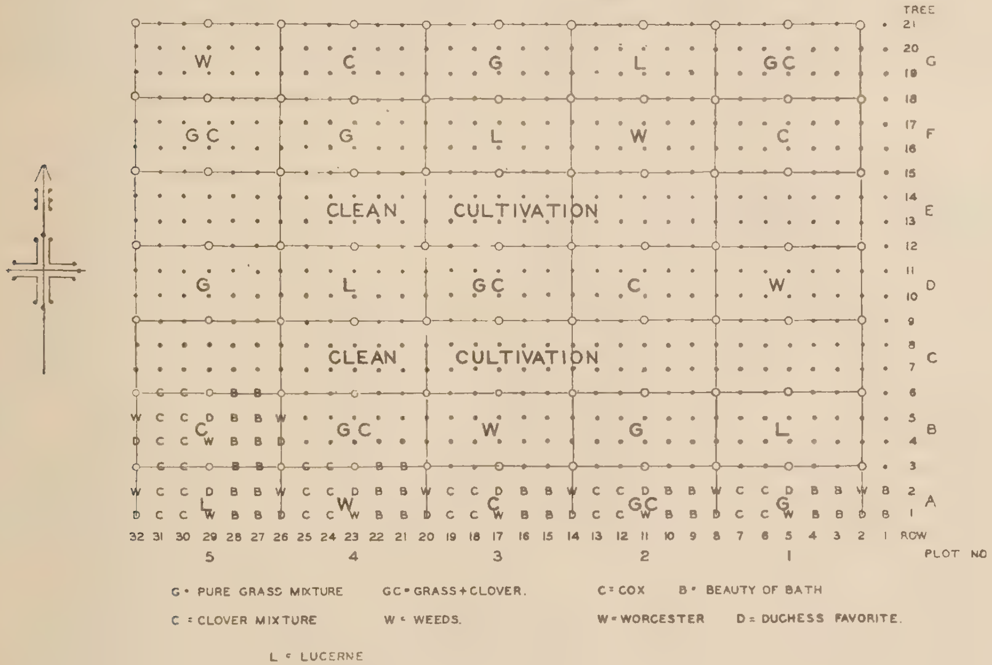

FIG. 1.
Plan of cover crop experiment 1940-43.

No cutting was done during 1940 while the various cover crops were establishing themselves, apart from mowing down the annual weeds which grew on all plots. During the second year, 1941, the cover crops were cut four times and the cut material was allowed to lie and rot where it fell. The yield of herbage from sample strips, each the length of a sub-plot and 3 ft. wide, chosen at random from each plot, was weighed and the cut material was redistributed, after taking small samples of it for the determination of percentage of dry matter. A fifth and final cut was made in May, 1942, after which all the cover crops were disced in. The clean cultivated strips were kept practically free from weeds throughout the whole period, by disc cultivation. After the turning in of the cover crops, small patches of them remained or sprang up again around the bases of the trees. These were gradually eliminated in the following years. From May, 1942, onwards all the plots had normal routine cultivations, which involved leaving the weeds untouched from the autumn to the following spring.

COVER CROP YIELDS.

Fresh and dry matter produced.

The kinds and amounts of seeds used are shown in Table I, together with the fresh and dry weights of the cut crops obtained in 1941 and the spring of 1942. From this it will be seen that the clover, grass + clover and lucerne plots gave by far the heaviest yields, each producing

TABLE I.

Kinds and amounts of seed used, and weights (tons/acre) of material produced.

Cover crop.	Kind of seed.	Amount of seed lb./acre.	1941.		1942.		Total.	
			Fresh.	Dry.	Fresh.	Dry.	Fresh.	Dry.
Grass	Perennial Ryegrass Italian Ryegrass	15·0 15·0	5·12	1·34	1·09	0·28	6·21	1·62
Clover	Broad Red Clover Late Red Clover	6·0 6·0	14·19	2·34	0·61	0·12	14·80	2·46
Grass+Clover ..	Perennial Ryegrass Italian Ryegrass Broad Red Clover Late Red Clover	7·5 7·5 3·0 3·0	12·04	2·42	2·45	0·54	14·49	2·96
Lucerne	Lucerne (alfalfa)	20·0	12·34	2·29	1·66	0·31	14·00	2·60
Weeds	Natural seeding	—	1·39	0·36	0·36	0·08	1·75	0·44

a total of over 14 tons of fresh material per acre over the two seasons. The yields in 1941 were, in fact, the highest in the whole series of experiments and exceeded those of permanent swards. The grass and the weed plots had a higher dry matter percentage than the others (25·3 per cent. as against 19·5 per cent.), but even this did not bring their total yield of dry matter up to that of the two legumes and the mixture grass + clover. The weeds provided a very weak cover throughout the experiment, and made only a meagre contribution to the organic matter of the soil.

It is worth noting that the grass + clover mixture gave a higher yield of dry matter than the plots of grass alone or of clover alone. Over the whole period the total yield of dry matter of the grass + clover mixture was 2·96 tons per acre, while the mean of the grass alone and clover alone plots was 2·04 tons per acre, a difference of 45 per cent. This is clearly due to the stimulating effect of the clover on the grass associated with it. The effect is most strikingly seen in the single cut of 1942, in which the yield of the grass + clover mixture was actually greater than the total of the grass alone and clover alone plots covering twice the area and sown with twice the amount of seed.

Proportion of root to top.

The figures given in Table I concern only the cut material, but the part below ground is also important. In order to gain information on this, two sample prisms of soil were taken from each of the five cover crops in April, 1942, for determination of the relative proportion of root and top, and to give some idea of root distribution. The top and four exposed sides of each prism were enclosed in a stout wire cage with sides 15″ and 10″ long and a depth of 12″ (Fig. 7, Plate III). The depth for the deep rooting lucerne was extended to 24″ by caging a second prism immediately below the first. The caged prisms were cut clear below, inverted, made secure

with a lid, placed in a stream and gently rocked from time to time to wash away the soil. The last soil particles were washed off under gentle pressure from a hose. After thorough washing the plants were divided into four portions for weighing, viz. the leaves, the crown (roughly 1″ to 2″ long—the height of mowing), the upper 6″ of the roots and the remainder of the roots. To these four portions was added a fifth, viz. roots which had broken loose during the washing process. The amount of loose roots obtained showed that this method of root isolation was imperfect, but it was the best available under wartime conditions and gave reasonably good results. The more refined washing method described by Pavlychenko (1937), of which the above is a simplified procedure, would be desirable in fuller studies. In addition to the prism washing, borings were made with an auger to test the maximum root depth of the various cover crops.

The results are assembled in Table II in which the figures are the means of two samples of each cover crop. It will be seen that, as well as the roots, the crowns of the plants (the parts remaining below the normal mowing level) add an appreciable amount of fresh matter to the soil when the cover crops are finally turned in.

TABLE II.

Fresh weights (grm.) of tops and roots, and maximum root depths of cover crops. (April, 1942.)

	Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.
Leaves and stems (above 1″)	45·6	65·4	95·6	106·5	11·0
Crown (ground level to 1″) ..	59·4	54·7	84·5	38·4	35·2
Total top	**105·0**	**120·1**	**180·1**	**144·9**	**46·2**
Roots 0-6″	112·2	56·9	119·0	47·5	51·1
,, 6-12″	11·7	8·5	8·7	14·0	0·9
,, 12-24″	—	—	—	15·9	—
Loose roots	23·3	5·4	24·1	5·3	23·7
Total roots	**147·2**	**70·8**	**151·8**	**82·7**	**75·7**
Ratio top/root	0·7	1·7	1·2	1·8	0·6
Maximum depth of roots ..	28″	24″	26″	36″+	13″

The figures show the proportion of roots to tops and the distribution in depth in the soil. Thus (1) the amount of roots in the upper 6″ was 65 to 75 per cent. of the total roots in all the crops except lucerne, where it was only 50 to 60 per cent. The lucerne roots which occupied the second foot in depth amounted to 20 per cent. of the total. (2) The greatest weight of roots was obtained from the grass + clover plots followed closely by the grass alone. (3) The grass and weed plots had more root than top at the time of sampling, but the mean root : top ratio for the five cover crops is very near 1 : 1. While this ratio may change at different periods of the year, it is clear that the amount of root produced is very large and must have a considerable effect on the soil. (4) Lucerne had by far the deepest roots, penetrating more than 3 ft. deep, while the weak grasses on the weeds plot had the shallowest roots.

The existence of the greater part of the roots in the upper 6″ of soil is in conformity with the results of soil moisture studies on these and other plots, in which it was found that though the cover crops dried the surface severely, there was relatively little difference between grass and clean cultivation plots at a depth of 18″ and lower in the early part of the year, though it became marked later (Rogers, 1937). Thus, the competing effect of certain cover crops for water, though important, is less in the deeper layers than might perhaps be expected. The amount of root below 6″ in the grass, clover and weeds plots was remarkably small as compared with the amount above 6″. Apple roots at Malling were found to go markedly deeper, however

(Rogers and Vyvyan, 1934). About 70 per cent. of the fibrous roots of trees on rootstock No. IX were more than 12″ deep, and vertical roots descended right to the underlying rock.

Soil analyses.

A series of chemical analyses of the soil were kindly made by Dr. N. H. Pizer of Wye College. The soil samples were taken a month after the cover crops had been disced in, and from four depths, 0-6″, 6-12″, 12-18″, and 18-24″. Each sample was composed of four borings, and samples were taken from each of three replications of each cover crop.

The figures for organic matter per cent. of air dry soil (by Walkley's method), for available potassium and phosphorus, in parts per million in the soil extract (by a modified Morgan method), and the pH values (by the glass electrode method) are given in Table III. They show that under all cover crops the soils have higher organic matter contents than under clean cultivation,

TABLE III.

Chemical analyses of soils one month after cover crops turned in. (June, 1942.)

Organic matter (% air dried soil).	Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.	Cultivation.
0- 6″	2·63	2·73	2·57	2·57	2·53	2·41
6-12″	1·53	1·43	1·40	1·40	1·30	1·28
12-18″	0·90	0·97	0·97	1·00	0·90	0·92
18-24″	0·80	0·77	0·73	0·80	0·87	0·82
Mean	1·46	1·47	1·42	1·44	1·40	1·36
Available K (p.p.m. in extract).						
0- 6″	27·7	26·7	21·3	26·7	24·7	23·7
6-12″	6·7	8·7	7·0	6·0	4·0	6·2
12-18″	4·0	4·1	4·0	2·7	2·7	3·2
18-24″	3·3	3·3	2·1	2·0	1·9	2·6
Mean	10·4	10·7	8·6	9·3	8·3	8·9
Available P (p.p.m. in extract).						
0- 6″	5·83	5·73	4·87	5·53	5·40	5·28
6-12″	1·43	1·30	1·67	1·30	0·93	1·08
12-18″	0·33	0·40	0·47	0·63	0·47	0·47
18-24″	0·20	0·20	0·27	0·27	0·37	0·20
Mean	1·95	1·91	1·82	1·93	1·79	1·76
pH.						
0- 6″	7·23	7·23	7·33	7·63	7·33	7·33
6-12″	7·37	7·43	7·43	7·57	7·50	7·50
12-18″	7·50	7·37	7·43	7·60	7·23	7·46
18-24″	7·30	7·47	7·33	7·53	7·70	7·48
Mean	7·35	7·37	7·38	7·58	7·44	7·44

especially in the top 12″. This increase is considerable, amounting on the average to 3·2 tons of dry organic matter per acre, assuming that a cubic foot of soil weighs 100 lb. Among the various cover crops, grass, clover and lucerne stand high in this respect, with the weeds making the smallest addition of organic matter.

The pH figures in general are somewhat lower where the organic matter is higher, as might be expected ; the lucerne plot being an exception. The figures for available potassium and phosphorus are too variable for much comment, but in general they follow the same pattern as those for the organic matter.

EFFECT OF THE COVER CROPS ON TREE GROWTH.

The effect of the various cover crops on the growth of the trees was studied by measuring the girth of the trunks, 9″ above the union, each winter. The year's increase of girth was expressed as a percentage of the previous girth, as this tends to minimize errors due to any initial differences in size of tree and gives a measure of the rate of growth. Additional information was obtained from the weight of the prunings and from visual observations of extension growth of each tree.

Table IV shows the percentage girth increase of the trees each year from 1940, the year of cover crop sowing, to 1943, one year after turning in. Statistical analysis of the figures is

TABLE IV.

Percentage girth increase (mean per tree) of apple trees in plots under various cover crops.

Year.	Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.	Cultivated.	Sig. Diff. (P=·05)
COX'S ORANGE.							
1940. (Cover crops sown) ..	4·54	5·02	4·96	4·64	4·84	5·48	1·46
1941	3·30	4·70	3·38	4·50	5·62	7·48	0·88
1942 (Cover crops turned in)	6·64	4·32	6·04	6·16	5·28	5·32	0·99
1943	5·36	3·54	3·94	3·86	4·72	3·97	1·40
Sum of percentages ..	19·84	17·58	18·32	19·16	20·46	22·25	
BEAUTY OF BATH.							
1940 (Cover crops sown) ..	4·38	4·58	4·64	4·34	4·24	4·60	0·93
1941	4·70	5·64	4·84	5·50	6·22	8·41	1·57
1942 (Cover crops turned in)	7·04	6·70	7·50	6·52	6·30	5·06	1·02
1943	5·00	4·26	4·92	3·82	3·86	3·88	1·20
Sum of percentages ..	21·12	21·18	21·90	20·18	20·62	21·95	

rendered slightly difficult because the clean cultivated strips, which, for convenience in cultural operations, ran right through the plantation, are not strictly comparable with the cover cropped plots. However, since the significant difference between this and a cover crop is likely to be, if anything, slightly less than that between the cover crops themselves, the latter figure may be taken without any differences being wrongly judged genuine.

It will be seen that in 1940, the year of sowing, though the figures give an indication of a check by all cover crops, none of the differences is significant. In the following year, 1941, very large and significant differences are seen, however. With the variety Cox, the trees in the grass and the grass + clover plots made less than half the growth of those in the clean cultivated plots ; those in the lucerne and the clover plots made about two-thirds the growth of those in the clean cultivated plots. The trees in the weeds plots made significantly less growth than those in the clean cultivated ones but significantly more than those in the sown cover

crop plots. Exactly similar grouping is shown by the Beauty of Bath trees but here greater variability makes a larger difference necessary for significance.

The turning in of the cover crops in May, 1942, considerably changed the picture. For in this year both Cox (except on the clover plots) and Beauty of Bath made more growth on the cover cropped plots than on clean cultivation. The difference is statistically significant for all cover crops with Beauty of Bath and for the grass and lucerne crops with Cox. Cox trees on the grass + clover plots appreciably increased their girth while on clover alone the trees made less growth than those on clean cultivation.

It can be seen from Table IV and from Fig. 2 that those cover crops which checked the Cox trees most severely while the crops were growing, stimulated the growth most after they had

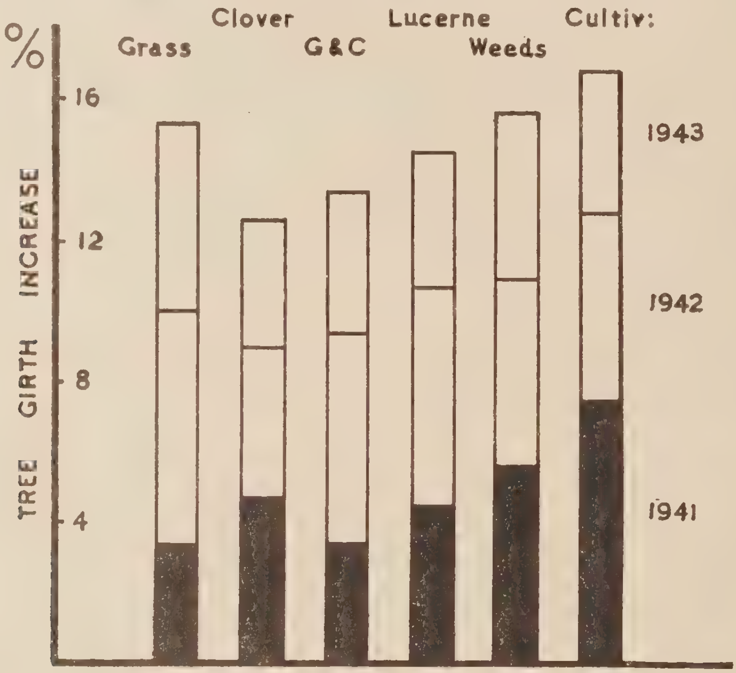

Fig. 2.
Percentage of girth increase of Cox's Orange Pippin, 1941-43. Black, during growth of cover crops ; white, after turning them in.

been turned in. Other cover crops like clover and weeds induced a more or less steady tree growth. It should be noted that Fig. 2 does not include the year 1940, in which no significant differences in tree growth appeared.

Though the total growth of the grassed trees for the years 1941, 1942 and 1943 did not exceed that of the trees on clean cultivation there are indications that with the continuing after-effects of the disced-in cover crops it will do so in 1944. This continued after-effect is perhaps shown even more clearly by the records of new wood cut off at pruning time, shown in Table V. They refer to current year's shoots only ; and since by the spur-pruning method adopted most of these shoots are cut off, they relate closely to the actual amount of new growth. As with the girth measurements, a very marked check in tree growth from all cover crops except the weeds one is shown in the second year, 1941. All trees in cover crops had significantly less new wood than those in clean cultivation. Furthermore, the trees in the grass + clover crop had a significantly smaller weight of prunings than those in the clover, lucerne and grass

plots, the trees in which, in their turn, had significantly less than those in the weed plots. The differences are larger in weight than in number of prunings, showing that the individual shoots of the trees in the cover cropped plots were shorter as well as fewer in this year.

TABLE V.

Number and weight (lb.) of prunings (new wood). Mean per tree. Cox and Beauty of Bath.

Year.		Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.	Cultivated.	Sig. Diff. for weight (P=·05).
1940	Weight ..	0·49	0·30	0·33	0·26	0·32	0·57	0·18
	Number ..	118	76	88	70	85	116	
1941	Weight ..	0·15	0·31	0·09	0·24	0·55	1·08	0·17
	Number ..	67	82	50	·73	109	157	
1942	Weight ..	0·93	0·85	0·72	0·77	0·92	0·86	0·34
	Number ..	111	111	96	112	112	120	
1943	Weight ..	1·50	0·56	1·21	0·49	0·79	0·52	0·30
	Number ..	139	57	111	60	86	58	

The turning in of the cover crops resulted in an immediate increase of tree growth, but in the first year of turning in this was only just about equal to that of the trees in the clean cultivated plots. Larger effects appeared in the second year. The trees in the grass and the grass + clover plots then (1943) had nearly three times as much weight, and between two and three times the number of new shoots as those in clean cultivation. This effect was so striking that these trees could easily be distinguished from the others in the various cover crop plots by the greater number and length of their shoots (Figs. 3 and 4, Plate I). On the other hand, the effects of the lucerne, clover and weed cover crops in 1943 were not large or significant.

It will be noted that the cover crops containing grass caused the greatest check whilst they were actually growing, and the greatest stimulus after they had been turned in. The effect of the clover and lucerne cover crops was milder in both respects. The relatively light check caused by lucerne is especially interesting in view of its deep root system and the fact that it was found to have a rather severe checking effect in the experiments of West and Howard (1938).

EFFECT ON TIME OF BLOSSOMING.

In early May, 1942, before the discing-in of the cover crops, the trees on the grass plots had hardly any open flowers whilst those on clean cultivation had many. Visual records of the estimated percentages of open flowers gave the figures shown in Table VI. Since the data refer to percentages, they have been transformed into Bliss's variate for purposes of analysis ($\text{Sin}^2\ \theta=$ proportion. Bliss, 1937 and 1938).

The retardation in blossoming in the spring of 1942 was very marked in the trees on the grass and the grass + clover plots, and actually made the full blossom period six to eight days later on these plots than on the clean cultivated ones. There was no marked difference in the trees on the clover and the lucerne cover crops.

Similar observations made in April, 1943, showed no marked differences in the time of blossoming of Beauty of Bath, but Cox trees had their blossoming significantly advanced wherever the cover crops had been turned in. The trees on the grass and the grass + clover plots, which were so lagging in the time of blossoming just before the turning in of the cover crops in May, 1942, headed the list in 1943 with a significant difference from the trees on the rest of the cover crop plots.

This result fits in with the findings of Wallace (1925) that variation in blossoming time is connected with the supply of available nitrogen. This nutrient was certainly relatively deficient in the grass and the grass + clover plots in May, 1942 ; but in the following spring, when the turned-in grass had had 11 months in which to rot down, it had become substantial in amount, and was already exerting a marked effect on the growth of the trees. Wallace's work (1925)

TABLE VI.

Percentage of flowers open. Cox and Beauty of Bath. (Mean per tree.)

Date.	Grass.	Grass + Clover.	Weeds.	Clover.	Lucerne.	Cultivated.	Sig. diff. (P=·05).
Cox.							
May 11th, 1942 (before turning in)	3	7	15	21	21	23	
Bliss transformation	*7·3*	*14·7*	*22·2*	*27·1*	*27·1*	*28·7*	*6·1*
April 20th, 1943 (11 months after turning in)	66	72	46	52	51	29	
Bliss transformation	*54·6*	*58·2*	*42·6*	*46·0*	*45·8*	*32·2*	*9·3*
BEAUTY OF BATH.							
May 6th, 1942	8	10	22	29	32	30	
Bliss transformation	*15·8*	*17·9*	*27·7*	*32·6*	*33·0*	*32·8*	*17·8*

showed that in pot culture omission of nitrogen considerably delayed the opening of the blossom buds.

EFFECT ON LEAF COLOUR.

No conspicuous colour differences in the leaves of the trees on the various cover crop plots were apparent until 1942, although in other experiments colour contrasts have been seen after a shorter period. In 1942 the leaves of the Worcester Pearmain trees on the pure grass plots had a yellow-green colour. The leaves of the trees on the other cover crop plots had the same healthy dark green colour as those of the trees on the clean cultivation plots. During 1943 no differences in colour could be observed in the leaves of the trees on the various plots.

EFFECT ON LEAF SCORCH.

Two types of leaf scorch have been shown to be closely connected with mineral deficiencies. Wallace and Proebsting (1933) and Wallace (1939, 1943), Kidson *et al.* (1940) and others have shown a connection between potash deficiency and marginal leaf scorch, and between magnesium deficiency and interveinal leaf scorch. The incidence of leaf scorch has also been found to vary with rootstock and variety (Hatton and Grubb, 1925 ; Hoblyn, 1941) and it also varies with seasonal conditions.

In the present experiment no appreciable marginal scorch occurred ; but severe interveinal leaf scorch appeared in the trees on certain plots in 1941, and very striking contrasts were noted. Interveinal scorch is characterized by browning and desiccation of the areas between the veins, usually towards the centre of the leaf. It becomes visible from July onwards and is associated with premature leaf fall progressing from the base of new shoots upwards. An excellent coloured illustration of it was given by Wallace (1943, Plate 42). This interveinal scorch has not been associated at East Malling with such serious effects on tree growth and cropping as are found there with marginal scorch, but the loss of effective leaf area and the premature defoliation cannot but have an unfavourable effect. Severe effects have been reported both by Wallace (1939) and Kidson *et al.* (1940) in certain cases.

In this experiment the amount of interveinal scorch was recorded by a visual method, using five categories representing percentages of leaves affected in equal steps of 20 per cent. The results for Cox in 1941 and 1942 are given in Table VII ; the amount of interveinal scorch in 1943 was negligible. The results with Beauty of Bath were similar but less contrasting. For analysis purposes the category values have been transformed to Bliss's variate (Bliss, 1937, 1938).

TABLE VII.

Interveinal scorch. Cox's Orange Pippin. (Mean per tree.)

	Grass.	Grass + Clover.	Lucerne.	Clover.	Weeds.	Cultivated.	Sig. diff. (P=·05)
Mean category value, 1941	0·35	0·68	2·63	3·67	4·15	4·55	
Bliss transformation ..	*14·26*	*20·50*	*40·08*	*59·46*	*66·12*	*73·97*	*20·14*
Mean category value, 1942	1·50	1·11	0·44	0·50	1·10	1·28	
Bliss transformation ..	*32·96*	*27·92*	*15·08*	*18·08*	*27·16*	*29·96*	*9·59*

Table VII shows that in 1941 the trees with grass below them, i.e. those on the grass and the grass + clover plots, had comparatively little interveinal scorch (about 10 per cent. of the leaves affected), while the trees on the clean cultivated plots had much (about 90 per cent. of the leaves affected). The trees on the clover and lucerne plots had intermediate amounts and these were all significantly less than those on the trees on the clean cultivated plots.

It was originally planned that leaf samples from the apple trees should be analysed each year by the Biochemical Section of the Research Station and samples were taken in September, 1941. Owing to pressure of other work there the analyses have not yet been completed, but preliminary results suggest that the cover crops have affected the mineral composition of the trees. It is hoped to publish these results when they are complete.

It may be interesting, however, to consider the leaf scorch results further, in the light of the known effect of potassium and magnesium on the occurrence of interveinal scorch. Wallace (1939), Kidson *et al.* (1940) and Garner *et al.* (1923) all found that the effect of magnesium deficiency was more severe in the presence of a relatively high level of potassium. Pearce and Appleman (1943) also have recently shown that the stems and leaves of certain grasses contained more K than those of certain legumes, though both contained about the same amount of Mg. If this may be taken as applying to the grasses and legumes used in the present trial, it will be evident that the grasses, by absorbing more K as they grew, would create a lower K : Mg balance, and thus have a more favourable effect on lessening interveinal scorch than legumes, under conditions where it would normally occur. On turning in the cover crops, however, both the K and the Mg which they had extracted from the soil would presumably become available to the trees ; and the grass, having absorbed more K than the legumes, would also release more in relation to its Mg, thus tending to have a less favourable effect than the legumes when turned in. Both the grasses and the legumes, it may be noted, lessened interveinal scorch, as compared with clean cultivation, over the whole period of the experiment. There are also indications that nitrogen may be playing an important part in the interrelations of K and Mg in the cover crops ; but more observations are needed before definite conclusions can be drawn.

The results certainly strengthen the evidence that suitable cover crops can extract from the soil and make available to the trees certain nutrients which were originally present in the soil but were there in an unavailable or not suitably balanced form for use by the trees. The grasses appear to have a more potent effect in relation to their bulk and weight than the legumes —a matter which deserves further study.

Similar contrasts were shown by records of leader defoliation, which need not be presented here in detail. Figures 5 and 6, Plate II, show clearly the contrast between trees on a clover cover crop and clean cultivation plot respectively.

While the most complete control of interveinal scorch was given by the two cover crops containing ryegrass (which were significantly better than any others) there was also an interesting contrast between the trees on the grass alone and the grass + clover cover crops. Their foliage on the grass alone plots, while the freest from interveinal scorch, was definitely pale and nitrogen-deficient, while that of the trees on the grass + clover plots was of a normal green colour as well as being practically free from scorch. The foliage of the trees on the clover and the lucerne plots was also of a normal green colour—suggesting that all the leguminous crops were providing at least sufficient nitrogen to prevent the appearance of starvation symptoms in the apple tree leaves.

Thus, in 1941, all the cover crops, but especially those containing ryegrass, showed a favourable effect in mitigating interveinal scorch, and the grass + clover crop induced the healthiest looking apple foliage.

In 1942 there was much less interveinal scorch on the trees in the plots in general, those in the clean-cultivated plots having only 15 per cent. of their leaves affected as compared with 90 per cent. in 1941. This can be due only to seasonal effects, since other factors were the same in both years. The trees on the grass, grass + clover, and weeds plots turned in in May, 1942, had almost the same amount of leaf scorch as those on the clean cultivated ones ; but the trees on the clover and lucerne plots had less, and owing to the greater uniformity of the trees on the plots in this season the difference, though less, is significant. While the leaf scorch in 1942 was nowhere serious, it is interesting to note that the beneficial effect of the grass cover crops was greatest while they were still growing, and that of the pure legumes occurred after they had been turned in. On the trees in the grass plots, in fact, there was actually a small, but unimportant, increase of interveinal scorch when the crops had been turned in. As already mentioned, the amount of interveinal scorch in 1943 was negligible.

Yield of fruit.

Cropping records for all four apple varieties were made each year, and are given as weight per tree in Table VIII. It is noteworthy that no very striking differences appeared till 1943. In 1940, when the cover crops were establishing themselves, they had no significant effect on weight of fruit. The Worcester Pearmain crop figures in 1941 are especially valuable, for the fruiting of the other three varieties was prevented by frost. In this year the Worcesters produced a smaller weight of fruit on all the cover-cropped plots than on the clean cultivated ones. The difference does not reach significance but is at least suggestive, for this was the year of greatest check to tree growth. In 1942 all four varieties yielded fruit, but there were no significant differences between them. Apparently neither the check due to the previous year's cover crops nor the stimulation due to ploughing in the crops in May materially affected the yield. It is possible that the two effects counteracted each other to some extent.

By 1943, however, the yield of fruit by the Cox trees on all the turned-in cover crops was heavier than that of the trees on the clean cultivated plots, though the difference is not quite significant. With Beauty of Bath trees on the grass cover crops the yield of fruit was significantly better than that of the trees on the clean cultivation plots, and the yields from the trees on the grass, grass + clover, and weeds plots were significantly better than those from the trees on the clover and the lucerne plots. The yields of the Worcester trees on all but two, and of the Duchess' trees on all of the cover cropped plots were heavier than those from the trees on the clean cultivated plots. Some of the increases in Duchess' are quite substantial ; but unfortunately, owing to the variability of the single trees of these varieties on each plot, the differences are not significant.

The total fruit yields over the four-yield period for each variety do not differ significantly between trees on cover crop and on clean cultivation plots except that of Beauty of Bath. This variety on the weeds cover crop plot yielded nearly significantly better than on the clean cultivation plot, and on the grass + clover plots almost as well. The yield of this variety on the clover plots was significantly worse than on grass, grass + clover and weeds plots. But the yields of Cox and Duchess' trees were better on the clover plots than on the grass plots.

TABLE VIII.

Total fruit yields in lb. (Mean per tree.)

Year.	Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.	Cultivated.	Sig. Diff. (P = ·05).
Cox.							
1940*	25·7	24·5	26·2	22·5	28·5	27·6	Not Sig.
1941	1·2	0·6	1·3	0·6	0·5	0·4	,,
1942	29·4	45·7	41·3	40·9	47·1	38·9	,,
1943	31·6	32·8	31·8	27·4	34·3	26·2	10·7
Total	87·9	103·6	100·6	91·4	110·4	93·1	Not. Sig.
BEAUTY OF BATH.							
1940*	20·7	16·5	21·2	20·3	21·7	21·2	Not. Sig.
1941	4·1	5·4	5·8	6·2	6·9	3·4	,,
1942	19·7	14·6	23·4	18·1	22·7	17·2	,,
1943	49·5	36·8	45·7	39·3	45·6	43·1	6·1
Total	94·0	73·3	96·1	83·9	96·9	84·9	13·2
WORCESTER.							
1940*	28·4	24·6	20·3	20·7	18·7	22·4	Not. Sig.
1941	18·7	17·6	14·3	13·9	19·3	21·1	,,
1942	19·7	24·6	17·0	27·3	22·7	26·8	,,
1943	47·5	35·5	36·2	32·0	32·3	32·9	,,
Total	114·3	102·3	87·8	93·9	93·0	103·2	,,
DUCHESS' FAVOURITE.							
1940*	24·0	27·3	22·4	24·1	24·3	21·3	Not. Sig.
1941	0·2	0·3	0·4	0·8	0·3	0·1	,,
1942	24·1	31·2	30·0	36·1	28·3	26·7	,,
1943	41·8	42·4	46·2	45·9	34·7	35·8	,,
Total	90·1	101·2	99·0	106·9	87·6	83·9	..
Total (all varieties)	**386·3**	**380·4**	**383·5**	**376·1**	**387·9**	**365·1**	Not Sig

* The weight of apples in 1940 was calculated from their recorded numbers on the trees.

Worcester seems to have withstood being grown on the grass plots better than the three other varieties; and Duchess' responded particularly well when grown on the legume crop plots. These differences, however, can be regarded only as preliminary indications.

The combined total yields for all varieties are particularly interesting, for the trees on each of the cover crop plots have proved superior to those on the clean cultivated ones, which produced the lowest yield of all over the four years. The differences are not large and are not significant ; but at least it can be concluded that the growth of the cover crops, even with the small amount of manure given, has not reduced the fruit crop in general ; and there are indications that important increases may be obtainable.

The lack of significance of some of the differences is due to rather high variability in fruit yield from plot to plot. This is probably partly due to the youthfulness of the trees, but it also emphasizes the importance of having good replication of plots in such trials.

EFFECT ON FRUIT SIZE.

As regards fruit size as shown by means of weight of individual fruits (Table IX), there were appreciable differences from year to year, but it was fairly uniform amongst the various treatments within each year. Data for Cox and Beauty of Bath for 1941 are omitted, since the yields were too small for useful comparison. There is a definite suggestion with Worcester that the average size of fruit from the trees on the cover crop plots was, in general, diminished in this season; but the difference is not significant. In the following years the size of fruit was nearly the same on the trees on all plots.

TABLE IX.

Fruit size as shown by mean weight., lb. per apple.

Year.	Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.	Cultivated.
Cox.						
1942	0·24	0·19	0·24	0·19	0·21	0·21
1943	0·17	0·16	0·17	0·16	0·16	0·17
BEAUTY OF BATH.						
1942	0·17	0·16	0·16	0·14	0·16	0·15
1943	0·11	0·10	0·11	0·10	0·10	0·10
WORCESTER.						
1941	0·14	0·14	0·14	0·14	0·16	0·17
1942	0·21	0·20	0·21	0·20	0·23	0·21
1943	0·16	0·20	0·17	0·17	0·17	0·18

EFFECT ON FRUIT COLOUR.

The effect of the cover crops on fruit colour was studied chiefly on the varieties Beauty of Bath and Worcester Pearmain, which, being mainly red, give a better contrast than Cox. As it was not feasible to use any of the detailed methods of colour grading of the picked fruit (Rogers, 1927) a visual record of colour of the fruit on the tree was made just before picking time. The appearance of the fruit was classified in ten categories, (1) being 0-10 per cent. coloured, and (10), 90-100 per cent. coloured. The categories were intended to refer to area of reddening, rather than intensity of colour, and from the mean colour grade value (V) the mean percentage coloured has been calculated. (Percentage $= 10V - 5$.) At the same time a record of yellowness of ground colour was made, on a similar scale, (1) referring to entirely green ground colour, and (10) to entirely yellow. This is rather an estimate of intensity than of amount of colour and it does, in fact, account for much of the variation in intensity of red colour. The mean colour figures have been converted to percentages in the same way as those for reddening.

The results are given in Table X, and they show that all the cover crops improved the colour of the fruit, both in area of reddening and in ground colour. The fruits on the trees in the plots containing grass had the best colour—a bright red one occupying more than three-quarters of their surface in Worcester, and in Beauty of Bath producing a reddening of about half their surface, with a good yellowish ground colour. By contrast the fruits on the trees on the cultivated strips had less than one-third of their surface reddened and they possessed

a much greener ground colour. Colour records were also made in 1942 and 1943, but the figures show no marked differences. It seems that the effect of such cover crops on fruit colour diminishes after the crops are turned in. This confirms the view that fruit colour is closely associated with available nitrogen in the soil.

TABLE X.

Percentages of fruit surface reddened and intensity of yellowing of ground colour. (Worcester and Beauty of Bath, 1941.)

	Grass.	Clover.	Grass + Clover.	Lucerne.	Weeds.	Cultivation.
WORCESTER.						
Per cent. reddened ..	83	63	76	66	66	31
BEAUTY OF BATH.						
Per cent. reddened ..	49	31	47	44	33	25
Yellowing of ground colour	48	49	51	50	48	35

The general conclusion is that the cover crops, while adding much organic matter to the soil, have so far neither depressed nor increased the yield or size of the fruit, but that they all greatly improved its colour. There are already indications that the beneficial results of the turned-in cover crops are continuing into later years.

VARIETAL EFFECTS.

Though the varieties included early (Beauty of Bath), mid-season (Worcester and Duchess') and late ones (Cox), the indications of differential response to the cover crops do not suggest that earliness or lateness of ripening is the main cause of the differences, but further data are needed on this matter. Worcester proved particularly valuable in the frost year (1941); but the mean crop per tree in all plots in the four years was nearly as great with Cox (97·8 lbs. per tree) as with Worcester (99·8 lbs.); Beauty of Bath gave a mean crop of 87·9 lbs. and Duchess' one of 93·9 lbs. per tree.

CONCLUSIONS.

The experiments described, while far from giving a complete answer to the question, "Which is the ideal cover crop for any given conditions?", have revealed certain basic principles. Under Malling conditions soil organic matter can be maintained or even increased by short-term cover crops, but some check to tree growth must probably be expected during the growth of the cover crop. The choice of a suitable cover crop and of a correct manurial programme is of cardinal importance.

From the results of this trial it can provisionally be assumed that certain short-term cover crops are suitable for trees of good vigour, in areas of rainfall of 26″ and over, and soils over 2½ ft. deep. By choosing a leguminous cover crop, e.g. red clover or lucerne, a large amount of green matter can be added to the soil in a short time, while at the same time improving fruit quality, reducing interveinal leaf scorch but moderately checking tree growth. Both Early and Late Red Clover easily become established and form a thick sward for two years. Lucerne is of particular interest where its deep root system may help to break a hard pan. Ryegrasses

will carry most of these effects a step further (probably too far in checking vigour) while a mixture of ryegrasses and clovers will take an intermediate position, depending on the proportion of grasses to legumes.

The practice of leaving weeds to establish themselves for two years before discing-in is likely to give a rather uncertain sward, but it is possibly useful where but little bulk and minimum cost are desired. A weak two-year natural grass sward had a relatively small effect on the soil, but its effect on the tree appeared reasonably good. The use of weaker-growing grasses such as Rough- or Smooth-stalked Meadow-grass is worth considering, and they are included in a trial now proceeding.

The results of this experiment suggest that under English conditions where the application of heavy nitrogenous dressings has latterly been the exception rather than the rule in fruit plantations, a manurial programme much more generous than that which would suit trees under clean cultivation should be arranged for trees under a cover crop if nitrogen deficiency effects are to be avoided. Even the leguminous cover crops seem to need some manure while growing. The appearance of the trees should be the final guide, but the amount provisionally suggested over and above that which would be given to trees under clean cultivation, is the equivalent of 224 lbs. sulphate of ammonia per acre, per annum, for legumes, while the crop is growing, and 400 lbs. for grasses and grass mixtures. On turning in the crop the equivalent of about 112 to 224 lbs. of sulphate of ammonia appears to be sufficient. Nitro-chalk would probably be preferable on acid soils.

It may safely be premised from the results of later work that where bulk of organic matter with less risk of check to tree growth is desired certain *annual* cover crops will be useful, of which adventitious weeds left from about July to the following spring will certainly be the cheapest and will probably form a desirable minimum cover cropping programme in preference to continuous clean cultivation. On the other hand, where trees need a severer check than that given by short-term cover crops, or in high rainfall areas, *long-term grass and legume swards* or permanent grass mixtures may be desirable.

Perhaps the most interesting result of the experiment is that, even with the same modest amount of fertilizer as was given to the clean cultivated plots, the cover-cropped plots have produced a large amount of organic material, with beneficial results to the apple foliage, with improvement in fruit quality, and with undiminished and in some cases an increased total yield of fruit over the four-year period. The only material from outside added to the cover-cropped plots and not to the clean cultivated ones was the few pounds of seed sown.

Thus it appears that orchard cover cropping offers the possibility of bringing about a reorganization of the capital resources of the soil, so that it gives a higher dividend while at the same time its capital value is maintained or even increased. This is achieved by the adaptation of some of the principles of rotation of crops and ley-farming to the conditions of a fruit plantation.

The short-term cover crops described in this paper are not ideal for all purposes, and it is well to note that a cover crop wrongly chosen or wrongly treated can do harm. The growing of cover crops is not free from difficulties—but, in general, the results indicate that with the whole range of available cover crops soil organic matter can be maintained, tree growth regulated and benefits to tree health and performance obtained within quite wide limits of local conditions.

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SUMMARY.

1. The effect of five cover crops grown for two years and then turned in, are compared with those of clean cultivation in an apple plantation. The crops were (i) Perennial + Italian Ryegrass (*Lolium perenne* and *L. italicum*); (ii) Broad Red + Late Red Clovers (*Trifolium pratense*); (iii) Ryegrass and clover mixed; (iv) Lucerne (alfalfa, *Medicago sativa*); and (v) Natural weeds (mainly *Poa*). The tree varieties were Cox's Orange Pippin, Beauty of Bath, Worcester Pearmain and Duchess' Favourite, all on No. IX rootstock, and twelve years old when the crops were sown.

2. Large amounts of green material were produced in two years, varying from 14 tons per acre for lucerne, clover, and ryegrass + clover, to 1·75 tons for weeds. The clovers had a markedly stimulating effect on the growth of the grasses mixed with them.

3. Studies on the root system and of the top : root ratio of the cover crops showed that with an established cover crop the top : root ratio in April was from 0·6 : 1·0 to 1·8 : 1·0. Thus, besides the weight of top, a very considerable amount of organic matter was added to the soil by the roots, practically equal to the weight of top at the time of turning in.

4. During their growth the cover crops caused a check on tree growth, but when they were turned in they had a stimulating effect. Both the check and the stimulation varied with different cover crops, being greatest with the ryegrasses in each case. The cover-cropped trees had not quite caught up with the clean cultivated ones in two years following the turning-in of the cover crops.

The check was probably due to competition both for nutrients and water. Manures were deliberately withheld, except for 168 lb. of sulphate of ammonia per acre over the period of growth of the cover crops, a similar quantity when the crop was turned in, and 224 lb. the following year. The importance of adequate manuring both for cover crops and trees is evident.

5. The total weight of fruit for all four varieties over the four years of the experiment, was slightly, but not significantly, larger on the trees on all the cover crop plots than on the clean cultivated ones.

There were indications of a reduction in size of fruit during the growth of the most vigorous cover crops, and increases of fruit yield followed the turning-in of the cover crops in most cases; but few differences reached significance. Some differences in varietal response appeared.

While the cover crops were growing they greatly improved fruit colour, but there was no such effect after they had been turned in.

6. The cover crops markedly reduced interveinal leaf scorch of the apple leaves. The ryegrass and ryegrass + clover crops had the greatest effect, reducing it in one year to about 10 per cent., as compared with 90 per cent. for trees on the clean cultivated plots. Symptoms of nitrogen deficiency were marked in the leaves of the trees on grass alone, but were much less evident in those on grass + clover. The legume crops had less effect on interveinal scorch while they were growing, but a more beneficial one after turning in. The possible role of the K : Mg ratio in this effect is discussed.

7. It is concluded that the conditions under which similar short-term cover crops would be most suitable are: trees of good vigour, soil not less than 2½ ft. deep and rainfall not less than 26″ per annum. The possibilities of alternative cover crops, such as annuals, including autumn weeds, and long-term swards for different conditions, are noted.

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PLATE I.

Fig. 3.
Cox's Orange Pippin tree over a former grass + clover cover crop (November 19th, 1943). Note strong leader growth. Residues of the cover crop remain around the trunk.

Fig. 4.
Cox's Orange Pippin tree over clean cultivation (November 19th, 1943). Note less growth than in Fig. 3. The growth of autumn weeds around the trunk can be seen.

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FIG. 5.
Cox's Orange Pippin tree over a clover cover crop (September 13th, 1941).
Note absence of leader defoliation.

FIG. 6.
Cox's Orange Pippin tree over clean cultivation (September 13th, 1941).
Note severe leader defoliation.

PLATE III.

Fig. 7.

Soil prism partially isolated and caged, for root estimation of a cover crop (Ryegrass).

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FIELD TRIALS OF DICHLORO-DIPHENYL-TRICHLOROETHANE (D.D.T.) AGAINST THE RASPBERRY BEETLE (*BYTURUS TOMENTOSUS* FABR.)

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ALTHOUGH many accounts of the remarkable insecticidal properties of "D.D.T." (*para-para'* dichloro-diphenyl-trichloroethane or, more correctly, $\alpha\alpha$-bis(p-chlorophenyl)-$\beta\beta\beta$-trichloroethane) have appeared in the popular and technical press, very few reliable details of experimental results have yet been made known. Wiesmann (1942, 1943), working in Switzerland in conjunction with the firm of J. R. Geigy A.-G., of Basle, found in preliminary trials that "Gesarol", a proprietary preparation of D.D.T. (Geigy, 1942; Läuger, Martin and Müller, 1944), showed promise against a variety of plant pests including Codling Moth, Apple Blossom Weevil, Plum Sawfly, and Raspberry Beetle. Martin, Stringer and Wain (1944) have recently described laboratory tests against various cabbage caterpillars that confirmed the outstanding activity of D.D.T. as a stomach poison and contact insecticide. So far as is known, however, no complete account has yet appeared of field trials against plant pests in Great Britain. At the request of the Agricultural Research Council and in collaboration with the Geigy Colour Company Ltd., of Manchester, a series of trials was started at East Malling in 1943 to evaluate the field performance of D.D.T. in a proprietary form and in an alternative preparation. The results of one of these trials, that against the Raspberry Beetle (*Byturus tomentosus* Fabr.), have now been confirmed in a second season and are presented below.

Steer (1932, 1933, etc.) showed that the Raspberry Beetle can adequately be controlled by a Derris wash containing 0·005 per cent. rotenone; but while one spraying is sufficient on raspberries, a second application is usually necessary on loganberries. The standard recommendation, therefore, is to spray loganberries about the middle and the end of June, and raspberries at the later time. The object of the present investigation was to find out whether D.D.T. could satisfactorily replace a rotenone-containing product in this control programme. In Wiesmann's experiment (1943) a spray containing 1 per cent. of "Gesarol", applied to raspberries once before blossoming and again at full flower, very markedly reduced the number of adult beetles to be found on the plants. Only a very small sample of the berries was examined, but there was an obvious reduction in the infestation. It has since been disclosed by the manufacturers (in lit.) that "Gesarol" contains 5 per cent. of technical grade D.D.T., a statement that has been verified by extraction of a sample with benzene and identification of the isolated material. The concentration of D.D.T. in Wiesmann's 1 per cent. spray was thus 0·05 per cent.

The host material in the present field trials consisted of three rows of loganberries, each row being 370 ft. long and in two sections. Each of these six half-rows formed one block which was divided into six plots of five plants each. Because of the method of training in these rows, the plots were divided across the middle of a plant, i.e. each plot consisted of four whole plants and two halves. Six treatments were randomized among each set of six plots, there being thus six replicates of each treatment. The treatments were re-randomized in the same blocks for the 1944 trial.

Sprays used.

In each year the treatments comprised the following :—

A. 0·025 per cent. D.D.T. as an E.M.R.S. laboratory preparation containing 0·05 per cent. Agral 2 and 0·025 per cent. sulphite-lye.

B. 0·05 per cent. D.D.T. as an E.M.R.S. laboratory preparation containing 0·05 per cent. Agral 2 and 0·025 per cent. sulphite-lye.

C_{43} and C_{44}. 0·025 per cent. D.D.T. as 0·5 per cent. of a 5 per cent. proprietary preparation.

D_{43} and D_{44}. 0·05 per cent. D.D.T. as 1·0 per cent. of a 5 per cent. proprietary preparation.

E. 0·2 per cent. Lonchocarpus ground root, equivalent to approximately 0·011 per cent. rotenone, with 0·05 per cent. Agral 2.

F. No spray.

The Lonchocarpus was a commercial sample of ground root yielding on analysis 5·4 per cent. rotenone determined as carbon tetrachloride complex. To allow an ample margin it was used at the concentration recommended for Derris of approximately 2 per cent. rotenone content. The D.D.T. used in E.M.R.S. preparations was of Swiss origin and contained approximately 75 per cent. pure *p-p′* compound. It was ground in a mortar with sufficient of the sulphite-lye in 10 per cent. aqueous solution to make a thin cream, and the Agral 2 added. The mixture was then passed through a hand-operated emulsifier which produced a finely-divided, flocculent suspension that settled fairly quickly, but was readily redispersed. The machine was washed through with the rest of the sulphite-lye solution. The proprietary preparations were not exactly alike in the two years. In **1943** a product of Swiss origin was used. This contained 5 per cent. of D.D.T. on a base consisting of chalk and bentonite, together with a wetter of the Nekal type (an alkyl naphthalene sulphonate). It was very unsatisfactory in that it settled out from suspension so rapidly that its use in this trial was possible only because of the very efficient agitation available in the spraying machine. In **1944** a British product was used, again containing 5 per cent. of D.D.T. (Batch 36, 77 per cent. *p-p′* compound), but on a base of china clay with some bentonite, and containing an alkyl naphthalene sulphonate and a dinaphthylmethane sulphonate as auxiliaries. This year the spraying machine had to be used without its agitator, but after a thorough initial stirring all the washes were maintained in satisfactory suspension by the return flow.

1943 Trial.

Spraying.—In order to test the persistence of the spray deposits only one application of each wash was made, namely on June 15th. This is about the date normally recommended for the first spraying of loganberries, but the season was unusually early and the application corresponded more nearly to the normal second spraying. Spraying was done under good conditions ; about half a gallon of wash was used per plant and wetting was satisfactory in each case. There was a light shower very shortly after completion and heavier rain some four hours later.

Egg laying was in progress by June 7th, and larvae had entered some fruits by June 18th.

Picking and recording.—There was no sign of damage to the plants resulting from any of the treatments. Picking of the crop started on June 21st and continued to August 4th, fourteen pickings in all. Up to June 30th there was still a visible deposit on the berries in treatments C_{43} and D_{43} (the proprietary material), but this amounted to little more than a pronounced bloom in C_{43} and even the heavier deposit in D_{43} was not considered commercially disadvantageous. A sample of sixty berries was picked from each plot at each picking throughout the season. As nearly as possible twenty berries were picked from each of the three inner intersections of the plants, from the two sides of the row, approximately in proportion to the ripe fruit. A few samples from the earliest and the latest pickings had less than sixty berries. The number of infested berries in each sample was then counted, the total number of berries in the combined

samples from each treatment being nominally 360 at each picking, or 5,040 for the full season. The percentage of infested berries at each picking and for the total crop over the whole season is shown in Table I.

The first two or three pickings from the sprayed plots showed little or no reduction in the degree of infestation when compared with unsprayed plots. Subsequently the control improved in each case up to the end of picking, but was not at any time satisfactory.

TABLE I.

1943 *trial. Berries infested per cent.*

Date of Sample.	Treatment.					
	A. Lower E.M.R.S. Preparation.	B. Higher E.M.R.S. Preparation.	C_{43}. Lower Proprietary Preparation.	D_{43}. Higher Proprietary Preparation.	E. Lonchocarpus.	F. Unsprayed.
June 23 ..	48·2	54·7	54·7	59·0	45·0	56·1
,, 25 ..	49·2	46·1	51·9	48·6	45·0	58·6
,, 28 ..	50·0	43·3	46·9	43·1	36·7	65·3
,, 30 ..	46·1	46·9	48·6	38·3	43·9	69·4
July 2 ..	38·1	35·0	34·1	28·9	40·6	67·5
,, 5 ..	30·3	28·1	27·5	26·4	26·4	62·2
,, 7 ..	31·4	25·0	28·6	19·2	30·6	73·6
,, 9 ..	28·1	26·1	32·2	24·4	26·4	70·3
,, 12 ..	23·1	12·8	16·1	11·4	15·6	66·9
,, 15 ..	23·1	24·4	21·9	20·6	20·6	61·7
,, 20 ..	14·0	16·7	20·0	15·0	19·7	55·3
,, 26 ..	11·7	9·7	11·1	9·4	10·8	50·0
,, 30 ..	6·2	6·5	11·4	6·7	5·1	38·1
August 4 ..	6·9	6·3	15·1	9·2	7·0	25·6
Total crop ..	29·4	27·4	30·9	25·9	26·9	58·8

The data for the whole crop were examined statistically by transforming the percentages for each replicate of each treatment to "Angles of Equal Information" (Bliss, 1937) and carrying out an analysis of variance on the resulting figures. This procedure was considered advisable in order to equalize the variances throughout, in view of the wide difference in percentage infestation between the unsprayed control and the treated plots. The result of this analysis is shown, in angular notation, together with the mean percentage reduction in infestation, in Table II.

The analysis of variance showed that the difference between treated and control plots was highly significant, but there was no significant difference between treatments. Although the

TABLE II.

Summary of 1943 *trial.*

Treatment.	Mean infestation (angular).	Mean reduction in infestation (per cent.).
A. Lower E.M.R.S.	32·8	50·0
B. Higher E.M.R.S.	31·6	53·4
C_{43}. Lower proprietary	33·2	47·4
D_{43}. Higher proprietary	30·4	56·0
E. Lonchocarpus	31·2	54·3
F. Control	50·4	
Significant difference (P=0·05)	4·7	

higher concentrations (treatments B and D) appeared to give slightly better control than the lower concentrations (A and C), further examination of the treatment variance apportionate to this part of the experiment still failed to show any significance.

1944 TRIAL.

Spraying.—In this season two applications of each wash were made, on June 16th and 30th, i.e. according to the standard recommendation. The sprays were applied as in 1943, though in 1944 the agitator in the machine was not working. Again about half a gallon of wash per plant was used at each spraying and wetting was good in each case. There was no rain for ten days following the first spraying; light showers occurred about twelve hours after the second and there was an unusually heavy thunderstorm (1·2 in. rain) three days later.

Beetles were abundant from May 24th and, intermittently, onwards, but eggs were difficult to find. On July 4th there were half-grown larvae in the plugs of the fruits.

Picking and recording.—There was again no sign of phytotoxic action. The procedure for picking followed exactly that of 1943. Picking started on July 4th and continued to August 18th, thirteen pickings in all. The fruit from the first picking of treatment C_{44} (lower proprietary) and that from the first three pickings of treatment D_{44} (higher proprietary) was judged commercially unsaleable because of the deposit on the berries. Some samples, particularly in the eighth, ninth, and tenth pickings, had less than sixty berries, and the total number of berries examined in each treatment for the full season, nominally 4,680, varied from 4,298 to 4,434. The percentage of infested berries at each picking and for the total crop over the whole season is shown in Table III.

TABLE III.

1944 *trial. Berries infested per cent.*

Date of Sample.	Treatment.					
	A. Lower E.M.R.S. Preparation.	B. Higher E.M.R.S. Preparation.	C_{44}. Lower Proprietary Preparation.	D_{44}. Higher Proprietary Preparation.	E. Lonchocarpus.	F. Unsprayed.
July 4 ..	5·7	6·1	6·1	6·1	6·1	25·6
,, 7 ..	3·9	3·3	5·3	7·2	11·0	23·9
,, 10 ..	3·6	1·7	4·7	4·2	2·2	24·7
,, 13 ..	1·1	2·5	3·1	2·8	3·6	18·6
,, 17 ..	1·1	1·4	1·4	1·9	1·7	17·5
,, 20 ..	1·1	1·1	1·9	1·9	0·6	18·3
,, 24 ..	2·9	2·2	3·7	1·7	5·5	26·1
,, 27 ..	1·7	2·5	1·9	2·4	2·0	31·9
August 2 ..	1·3	2·9	2·4	0·6	2·6	23·7
,, 4 ..	1·3	0·0	0·0	1·2	2·5	18·0
,, 9 ..	0·8	0·3	0·6	0·3	1·5	8·5
,, 14 ..	1·4	0·6	1·4	0·3	0·0	5·8
,, 18 ..	0·6	0·6	1·3	0·6	0·3	3·4
Total crop ..	2·1	1·9	2·7	2·4	3·1	19·2

Although a high degree of control by each spray treatment was evident in the first picking, the results were even better as the season progressed and were well sustained right to the end.

The data were again subjected to the angular transformation before analysis. The result, together with the mean reduction in infestation, is shown in Table IV.

The analysis of variance again revealed a highly significant difference between treated and control plots, but no significant difference between treatments. The apparent slight superiority

of the E.M.R.S. preparation over the proprietary and of the higher concentration over the lower were not found significant on further examination of the treatment variance.

Discussion of results.

The single spraying in 1943 was clearly inadequate to control the fairly heavy infestation, though this partial failure may have been accentuated by the rain that followed soon after spraying. It was evident, too, that the spraying was too late to affect the earliest larvae, whence the lack of control revealed in the earliest pickings. Nevertheless, D.D.T. in each preparation, and even at the lower concentration, compared favourably with Lonchocarpus in both the degree of control obtained and the persistence of the effect.

Table IV.

Summary of 1944 trial.

Treatment.	Mean infestation (angular).	Mean reduction in infestation (per cent.).
A. Lower E.M.R.S.	8·1	89·1
B. Higher E.M.R.S.	7·6	90·1
C_{44}. Lower proprietary	9·2	85·9
D_{44}. Higher proprietary	8·6	87·5
E. Lonchocarpus	9·4	83·9
F. Control	25·8	
Significant difference (P=0·05)	3·5	

In 1944 the infestation was very much lighter and the double spraying gave excellent control in every case. The 1943 result was fully confirmed in that all the D.D.T. treatments were at least as efficacious as Lonchocarpus.

The excessive spray deposit would militate against the use of the 1944 proprietary material, but this problem is not likely to be difficult to resolve. Further work may show that still lower concentrations of D.D.T. are effective, so reducing the amount of inert material applied. Alternatively an increase in the amount of D.D.T. in the spray concentrate is quite practicable, leading to the same end. Yet again, it may prove possible to develop a fluid concentrate from which inert solid diluent is absent. The E.M.R.S. preparation used in these trials was of this type, but would not be suitable for a commercial preparation since it was made up for immediate use and the problems of stability, storage, and the like were not taken into account.

SUMMARY.

αα-Bis(*p*-chlorophenyl)-βββ-trichloroethane (D.D.T.) has been compared with Lonchocarpus ground root in field trials against the Raspberry Beetle (*Byturus tomentosus* Fabr.). In each of two proprietary forms, one of which was of an unsatisfactory physical nature, and in a laboratory preparation, D.D.T. at 0·025 per cent. or at 0·05 per cent. gave as good control as a Lonchocarpus spray containing approximately 0·011 per cent. rotenone. The difference between treated and control plots was highly significant, but there was no significant difference in control as between either the products or the concentrations. In 1943 a single spraying with each one of the materials was only moderately effective against a fairly heavy infestation, whereas in 1944 double spraying gave an excellent control of a lighter infestation irrespective of the spray used. In no case was there any evidence of phytotoxicity. The proprietary material more satisfactory on physical grounds left a deposit on the fruit that made the earliest pickings unsaleable. Possible means of overcoming this disadvantage are suggested.

ACKNOWLEDGMENTS.

The author gratefully acknowledges the co-operation of the Geigy Colour Company Ltd., who supplied samples of D.D.T. and of the proprietary materials used, as well as technical data relating to these products. He is also indebted to Miss M. Bennett who did the spraying and supervised the picking, and to Messrs. T. N. Hoblyn and S. C. Pearce for most helpful discussion of the statistical treatment of the data.

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VIRUS INFECTION AND WATER LOSS IN TOMATO FOLIAGE

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Many of the symptoms of virus infection in plants are suggestive of excessive water loss from the tissues. In the tomato, detached Mosaic-infected leaves were found by Heuberger and Norton (1933) to lose water more rapidly than healthy leaves. These workers showed that infected plants transpired considerably less water than the controls for some time after symptoms appeared, but that gradually they transpired an amount of water approaching that of the controls, even though at this time they were considerably smaller than the latter. Thus, on an area or a dry weight basis, the infected plants tended to lose water at a higher rate than the controls. Using a weighing method, no effect of Mosaic virus on transpiratory losses from the whole plant could be detected during the incubation period. They pointed out that the effect of the virus on the transpiration of leaves that had developed before inoculation, which do not show symptoms later although they contain virus, was still not known.

Evidence of abnormal water relations has been observed repeatedly by the writer, in studies of virus infection in young tomato plants. Indeed in many cases the initial symptoms of infection appear to be induced directly by a deranged water balance in the plant. An attempt has therefore been made to follow transpiration in tomato leaves during the incubation period.

Methods.

Comparative rates of water loss from pairs of tomato leaflets attached to the *same* leaf stalk were measured, using the cobalt chloride paper method. Various modifications of the leaf clip described by Meyer (1927) were tried and the one finally adopted is shown in Fig. 1. It was not considered necessary to make the method quantitative for this preliminary study and attention was devoted to simplifying procedure to facilitate large numbers of readings under glasshouse conditions.

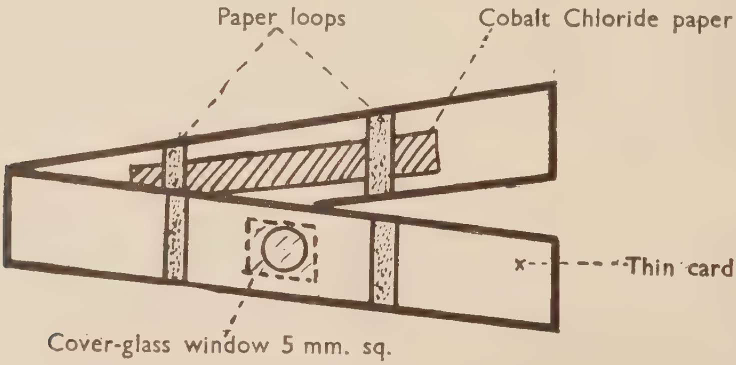

Fig. 1.
Cobalt chloride paper leaf "clip".

Celluloid clips were used in the earlier experiments but strips cut from thin postcards were found to be more suitable for holding in contact with thin leaves. It was necessary to hold the card on the leaf with the fingers, to prevent mechanical injury to the thin lamina and succulent veins of the young tomato leaflets. Only in this way was it found possible to make

repeated observations without causing permanent damage. The cobalt chloride paper was replaced when necessary by slipping a new piece under the paper loops. Separate clips were always used for healthy and infected leaves to prevent accidental infection of the controls. For rapid work in the glasshouse the cobalt chloride papers and the card clips were dried over a piece of sheet tin heated with a small spirit lamp.

EXPERIMENTAL RESULTS.

Experiments with the leaflets of young tomato plants were carried out to see whether the transpiration rates of twin leaflets were comparable and whether wiping the surface of a leaflet with a muslin pad soaked in the juice of a healthy tomato leaf produced an effect on water loss. Good agreement was found between twin leaflets and no marked differences could usually be detected between untreated leaflets and those inoculated with the juice of healthy leaves. To keep conditions comparable, the control leaflet was always wiped with a pad soaked in the juice of healthy leaves. This is perhaps important where sodium sulphite solution is added to the juice to facilitate the transference of a virus such as that which causes the Spotted Wilt disease.

The stage of development of the leaf, the variety of tomato and the water content or degree of "hardness" of the tissues were sometimes found to influence the results, and data concerning these factors have been included as far as possible.

The first experiment was made with a strain of tobacco Mosaic virus (Tomato Mosaic A17 (Selman, 1941)), and gave a clear indication that virus infection rapidly affected the rate of water loss from the leaf. The earlier experiments, carried out in the autumn of 1943, gave very consistent results, but subsequent work has shown that occasionally factors may intervene which obscure the general conclusion.

EXPERIMENT 1. INFLUENCE OF TOBACCO MOSAIC VIRUS ON TRANSPIRATION.

Plants : Tomato var. E.S.1 at the 12-13 leaf stage, in 3 in. pots.
Leaflets : Distal pair of 7th leaf from apex.
Control : Moistened with juice of healthy White Burley tobacco, upper surface only.
Infected : Inoculated with juice of White Burley containing the tobacco Mosaic virus strain, upper surface only.
Date : October 18th-November 1st, 1943. First reading taken immediately before inoculation.
Readings : Daily, in subdued light in laboratory, using celluloid clip.

The results assembled in Table I provide evidence of an increased rate of water loss from tomato leaflets on the third day after inoculation with tobacco Mosaic virus, from both upper and lower leaf surfaces.

TABLE I.

Time in minutes for $CoCl_2$ colour change.

Date :	Oct. 18.	19.	20.	21.	22.	25.	Nov. 1.
Upper surface—							
Control ..	8·5	8·0	8·5	7·0	6·0	7·0	4·0
Infected ..	8·5	8·5	7·0	6·0	5·0	3·5	3·0
Lower surface—							
Control ..	4·0	4·0	4·0	3·0	2·5	2·5	1·5
Infected ..	4·0	4·0	3·5	3·0	1·5	1·0	1·0

EXPERIMENT 2. INFLUENCE OF TOMATO SPOTTED WILT VIRUS ON TRANSPIRATION.

Plants : Six tomatoes, var. Potentate, at 6-leaf stage.
Leaflets : Distal pair of 4th leaf from apex in each plant.
Treatments :

	C	Uninoculated control.
Upper surface only	H	Inoculated with juice of healthy Arum leaf mixed with 0·5 per cent. sodium sulphite solution.
	SW	Inoculated with juice of Arum leaf containing Spotted Wilt virus, plus 0·5 per cent. sodium sulphite solution.

Readings : In laboratory using celluloid clip.

The following comparisons were made using twin leaflets :

C *v.* H, C *v.* C, SW *v.* C, SW *v.* H, SW *v.* SW, H *v.* H.

Colour changes of the cobalt chloride paper were noted to the nearest quarter minute.

The mean values for upper and lower surfaces are shown graphically in Fig. 2 and the complete data for two plants inoculated with Spotted Wilt virus are given in Table II.

TABLE II.

Time in minutes for $CoCl_2$ colour change.

Leaf surface.	Plant No.	Treatment.	Oct. 20.	21.	22.	23.	25.	27.	Nov. 1.
Upper ..	3	C	4·5	4·5	4·5	4·0	3·0	3·5	1·5
		SW	4·5	4·0	4·5	2·0	1·5	1·25	1·0
Lower ..		C	2·5	2·0	2·5	2·0	1·5	1·5	1·0
		SW	2·0	2·0	2·0	1·0	1·0	0·5	0·25
Upper ..	4	H	4·5	4·5	4·5	3·5	3·5	3·5	1·5
		SW	4·5	4·5	4·5	2·0	1·5	1·25	0·75
Lower ..		H	2·0	2·0	2·0	1·5	1·5	1·5	0·75
		SW	2·0	2·0	2·0	1·0	1·0	0·5	0·25

From the curves in Fig. 2 it will be clear that there was a marked rise in the rate of water loss from leaflets three days after inoculation with the Spotted Wilt virus. Twelve days after inoculation the rate of water loss from the control or healthy leaflets opposite to infected ones tended to increase. This may have been due to the migration of the virus into these leaflets from their partners. Plants which had shown this effect after inoculation were kept in the glasshouse for some weeks, but in no case did symptoms of Spotted Wilt disease appear. This apparent failure to transmit this virus to young tomato plants during the autumn and winter months has frequently been observed, particularly when infective juice has been taken from diseased Arums or Chrysanthemums. It would seem, therefore, that a transpiration method for detecting the presence of a virus might prove to be very much more sensitive and rapid, at certain times of the year, than waiting for an abnormal growth response to appear on indicator plants.

Experiments were continued during the winter of 1943-44 and very consistent results were obtained. An experiment started on March 22nd gave an inconclusive result as the relevant data given below will show.

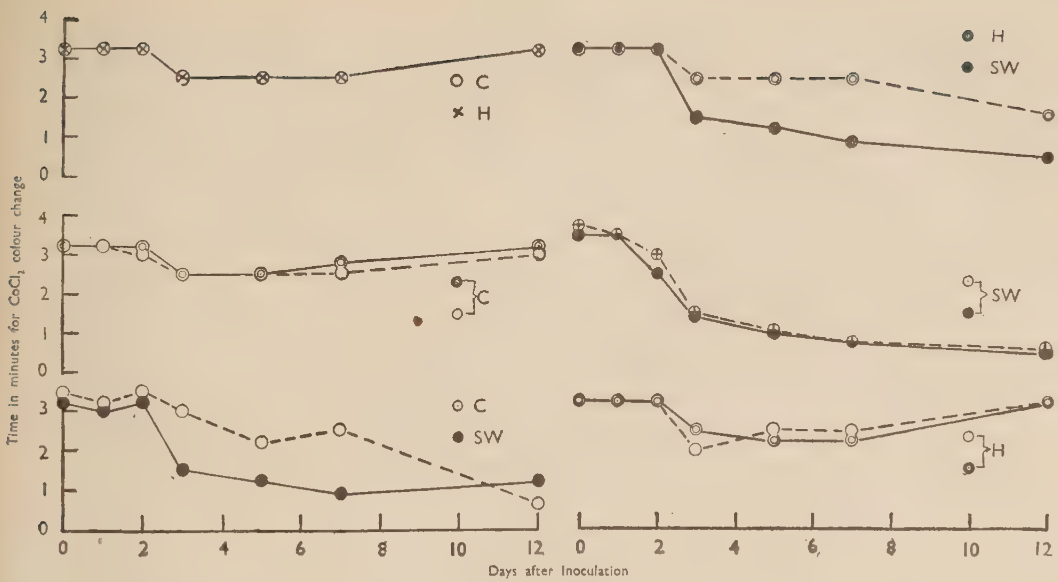

FIG. 2.

Twin leaflet comparisons of times for $CoCl_2$ colour change in tomato var. Potentate. Inoculated with Spotted Wilt virus (SW); inoculated with juice of virus-free leaves (H); uninoculated (C).

EXPERIMENT 3.

Plants: Tomatoes var. Potentate, growing in 6 in. pots. At 11th-12th leaf stage, soft but balanced growth; well supplied with water throughout. Leaves thin and of high water content.

Leaflets: Distal pairs on 3rd leaf from apex.

Inocula: Juice of tomato leaves, healthy and tobacco Mosaic-infected respectively.

Duration: March 22nd-April 3rd, 1944.

Readings: In glasshouse; upper surface of leaf inoculated; transpiration measured on lower surface only.

The data are shown graphically in Fig. 3. No effect of Mosaic virus on transpiration could be detected until the time of appearance of Mosaic mottling in the leaves. The method may

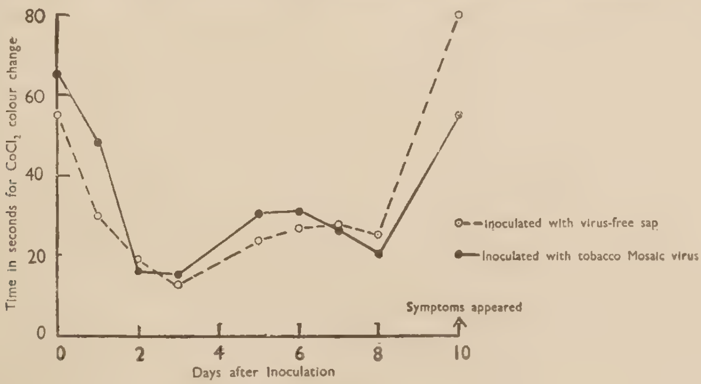

FIG. 3.

Tobacco Mosaic on soft plants of tomato var. Potentate.
Upper surface inoculated. Lower surface measured.

not be sufficiently sensitive to detect differences in the rates of water loss in leaves losing water very rapidly. The time for the colour change was less than 20 seconds on several occasions during this experiment, and errors in colour matching are relatively great over such short periods. Measurements made on the upper surface of leaves usually gave times ranging from one to eight minutes. Subsequent work with crop plants showed also that the cultural conditions under which different tomato varieties are grown has some effect on the transpiration response to Mosaic infection.

Experiment 4.

In view of the result obtained in the previous experiment a further trial with tobacco Mosaic virus was carried out, to see whether the effect was in part seasonal. In this case older plants and older leaves were used and also a different variety of tomato. The details are :

Plants : Two tomatoes, var. Ailsa Craig, at the 13th leaf stage, in 6 in. pots, growing in sterilized, unmanured, maiden loam. Lower leaves yellowing slightly.

Leaflets : Proximal pair of 6th and 8th leaves from apex.

Inocula : Juice of tomato leaves from healthy and tobacco Mosaic-infected plants respectively.

Duration : April 11th-17th, 1944.

Readings : In glasshouse, using card clips ; both upper and lower surfaces of leaf measured.

The original data are shown in Table III. Measurements for the upper surface were made to the nearest quarter minute, those for the lower to the nearest second.

Table III.

Time in minutes for colour change on upper surface.

Plant.	Leaf.	Time in hours after inoculation.						
		0.	23.	42.	67.	72.	90.	138.
A	6 H	5½	4½	4	6½	5½	5½	7
	TM	5¼	5	2	3¾	3½	4	7
	8 H	4¾	4½	3½	5	4	6¼	10
	TM	4¾	4½	2½	3¾	3	6	8½
B	6 H	3½	3¼	2¼	4	5	4	7½
	TM	3¼	3	1¼	2½	3	3½	7½
	8 H	2¾	2¾	2¾	6¼	6¾	4½	8
	TM	2¾	2¾	1¾	4¼	4¾	3¾	7½

Time in seconds for colour change on lower surface.

Plant.	Leaf.	0.	23.	42.	67.	72.	90.	138.
A	6 H	75	60	50	30	45	50	75
	TM	85	75	25	25	30	35	75
	8 H	65	90	40	30	45	60	85
	TM	65	90	30	45	45	40	80
B	6 H	55	45	40	75	30	22	70
	TM	45	45	30	45	25	33	65
	8 H	105	120	45	75	40	64	75
	TM	125	135	25	55	35	40	70

In Fig. 4 the mean values for upper and lower surfaces respectively are shown graphically. There was thus evidence that the transpiration rate had risen with virus infection after 42 hours. After 6 days the rate of water loss from the control leaflets approximated to that of the infected, which may indicate that the virus had invaded the control leaflet.

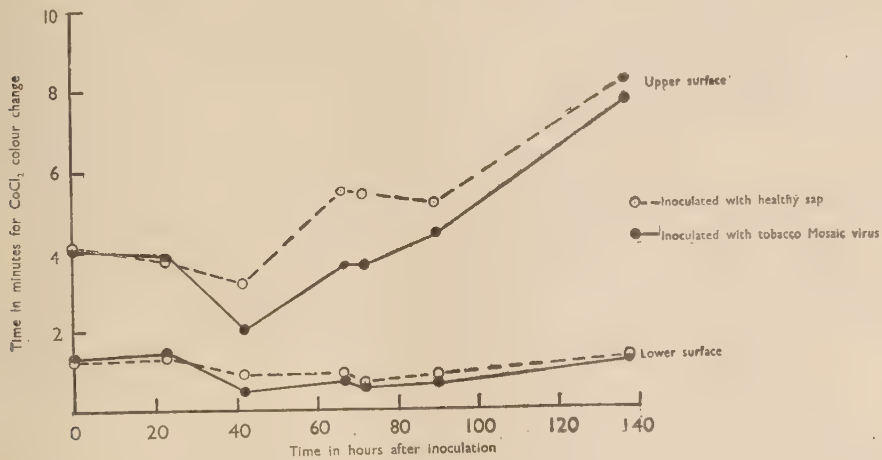

FIG. 4.

Tobacco Mosaic on tomato plants var. Ailsa Craig. Upper surface of leaf inoculated.

From the above data it may be seen that although the inoculum was placed on the upper side of the leaves the effect of the virus on water loss was evident from both upper and lower surfaces. It was considered possible that the virus might be affecting stomatal activity and the distribution of the stomata was therefore studied.

Epidermal strips were taken from leaves of plants similar to those used in Experiment 4. Stomata were abundant on the lower surface but were either absent or confined to the midrib on the upper surface of the leaves. Eckerson (1908) found 10-11 times as many stomata on the lower as on the upper surface of tomato leaves, and strips taken from fruiting plants on the nursery at Cheshunt did reveal a small number of stomata on the upper surface. Evidently the distribution of stomata must be related to the type of growth and age of the plant. Since transpiration was found to be initially increased by virus inoculation in Experiment 4 from leaf surfaces which contained few or no stomata, it was concluded that the main effect must be on cuticular transpiration. It may be noted that Krausche and Gilbert (1937) have concluded that transpiration in tomato seedlings is largely cuticular.

Effect of virus on leaves at different stages of development.

An indication of the influence of the position of the leaf on the plant on the virus-induced transpiratory effect was obtained by measuring transpiration on a single tomato plant at the 10th-11th leaf stage, using the distal pairs of leaflets on six leaves. The experiment was started on February 18th and continued for five days. Tomato Yellow Mosaic virus was compared with healthy tomato leaf sap. Fig. 5 gives a graphical summary of the data. Inoculations were made on the upper surface of the leaf: measurements were made on the lower surface only.

From Fig. 5 it may be seen that transpiration was increased rapidly on older leaves and the effect of the virus was less marked or absent with the younger leaves. This conclusion was confirmed by a similar experiment using a strain of tobacco Mosaic virus on plants of the var. Potentate at the 6th leaf stage.

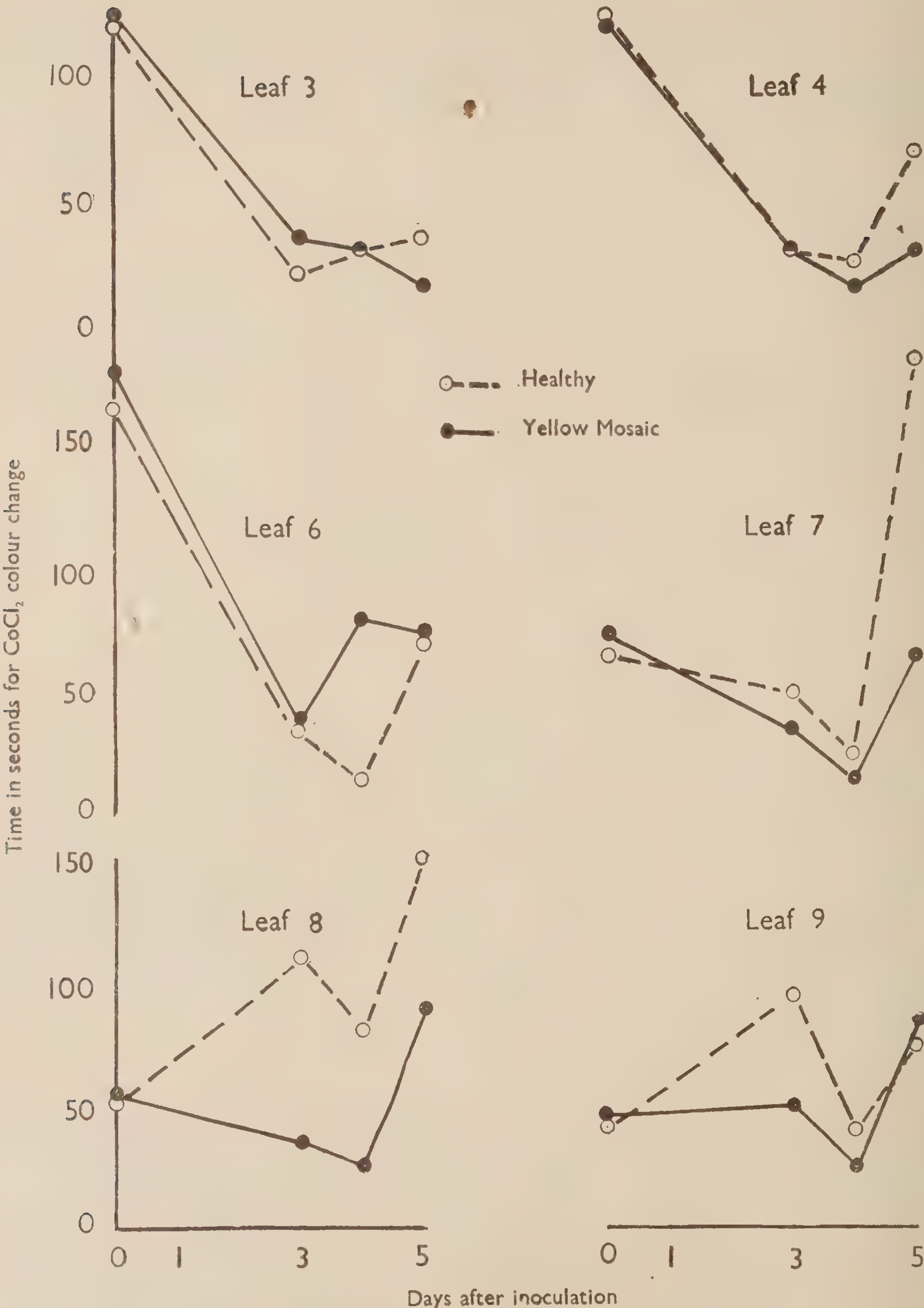

FIG. 5.

Effect of Yellow Mosaic virus on tomato leaves at various stages of development.

Water loss from the whole plant.

Weighing methods for the measurement of water loss from plants are less subject to error than the cobalt chloride paper method, yet Heuberger and Norton (1933) could find no effect of virus on transpiration during the incubation period by this method. If leaves of different ages react differently, as suggested by the experiment described above, this may explain a negative result with the whole plant. Inspection of Heuberger and Norton's graphs 1 and 2, however, shows that small changes were in fact recorded, although in their experiments the controls were not moistened with healthy tomato leaf sap. Several experiments were therefore carried out, in which whole plants were inoculated and water loss determined by weighing. One such experiment is described below.

Eight tomato seedlings, var. Potentate, at the 3rd-4th leaf stage, were planted in 100 ml. beakers in moist peat and the tops of the beakers covered with grease-proof paper and sealed with plasticine. The rate of water loss was determined for each plant during a preliminary period in the glasshouse. Four plants were then inoculated on all leaves with a strain of tobacco Mosaic virus and the remainder wiped with a pad soaked in healthy tomato leaf sap. The mean rates of water loss over a three-day period are shown in Table IV, together with the relative values taking the rates prior to inoculation as 100.

TABLE IV.

Date.	Transpiration period.	Grm. water/plant /hour. C	TM	Relative rates of water loss. C	TM
May 16	2.30-4.0 p.m. G .. .. Inoculated 5.0 p.m.	0·449	0·361	100	100
May 17	5.30 p.m.-9.0 a.m. L. ..	0·068	0·059	15	16
	9.0-10.0 a.m. L	0·423	0·295	94	82
	10.0-11.30 a.m. L	0·370	0·262	82	73
	11.30-2.30 p.m. G	0·393	0·283	87	78
	2.30-5.30 p.m. L	0·410	0·317	91	88
May 18	5.30 p.m.-9.0 a.m. G ..	0·133	0·103	30	29
	9.30 a.m.-5.30 p.m. G ..	0·563	0·474	125	131
May 19	5.30 p.m.-9.0 a.m. G ..	0·068	0·066	15	18

C=Controls inoculated with healthy tomato juice.
TM=Plants inoculated with tobacco (tomato) Mosaic virus A17.
G=In glasshouse. L=In laboratory.

There was thus an indication of a slight initial reduction in rate of water loss in the infected plants followed by a recovery or slight increase during the first few days of the incubation period, and this result is in agreement with the data figured by Heuberger and Norton, although not apparently considered by them to be of any significance.

DISCUSSION AND CONCLUSION.

That plant viruses may induce changes in the passage of water through infected tissues has been recognized in plants other than the tomato. Thus Krüger (1932) found that transpiration in potato plants affected with Leaf Roll may at times be increased, and at others decreased. With Mosaic infection of potatoes, Kuprewicz (1934) reported that transpiration is nearly doubled, and Kaho (1935) stated that the cells of virus-infected potato tubers are more permeable

to water than those of healthy ones. On the other hand, Mosaic-infected bean leaves transpire less, on an area or dry weight basis, than do healthy leaves, whereas infected pods lose water more rapidly than healthy pods of the same size (Harrison, 1935). The earlier work has not, however, been concerned with water loss during the incubation period. The fact that transpiration changes can be detected 42 hours after inoculation (in one experiment after only 20 hours) would appear to indicate that changes in the water relations may be one of the first reactions of living cells to the virus proteins. The primary effect of the virus appears to be upon cuticular transpiration in the tomato, and not upon stomatal behaviour.

SUMMARY.

1. Changes in the rates of water loss from twin tomato leaflets inoculated with the sap of healthy and virus-infected leaves have been studied during the incubation period, using the cobalt chloride paper method.

2. In leaflets inoculated with Spotted Wilt, tobacco Mosaic and tomato Yellow Mosaic viruses a marked increase in transpiration occurred 1-3 days after inoculation. It is suggested that transpiration methods could be used for the rapid detection of the presence of a virus at seasons when symptom appearance on indicator plants may be long delayed.

3. Inoculation of the upper leaf surface led to increased transpiration from both upper and lower surfaces.

4. The effect of the virus appears to be primarily on cuticular transpiration.

5. Leaves at various stages of development may react differently to virus inoculation. Older leaves tend to show the greatest increase in rate of water loss.

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THE CONTROL OF BACTERIAL CANKER AND LEAF-SPOT IN SWEET CHERRY

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By the end of 1934 certain eight-year-old standard Bigarreau de Schrecken cherry trees at East Malling Research Station—part of a rootstock trial of vegetatively propagated Mazzard* seedlings selected for their ease of rooting—had become so severely mutilated by the Bacterial Canker organism (*Pseudomonas mors-prunorum* Wormald) that the trial was seriously jeopardized. Other trees on the mixed plot were Early Rivers and Turkey Heart cherry, and Victoria and Czar plum, but these were at that time virtually unaffected. The best of the Schrecken trees were therefore used for a spraying trial, in which simple comparisons were made in order to develop a spray programme. From previous etiological studies (Wormald, 1937, 1938) it appeared that the problem was to ward off infection during the period of susceptibility from autumn to spring, when it can occur through wounds, especially, perhaps, leaf-scars.

Spraying Trials.

Progress reports on the results of the trials have already been published (Montgomery and Moore, 1935-38); what follows is a fuller account of them.

First Year, 1934-35. Bordeaux mixture was used at 10-15-100† in autumn and 8-12-100 in spring. The pre-blossom application was to prevent Leaf-Spot from becoming established on the earliest leaves, which are the sources of infection for the later ones.

Sixty-nine comparatively good Schrecken trees were selected for the trial; nine of these were unsprayed controls (**C**), and the remaining sixty formed three lots of twenty trees each, treated as follows:

A—Autumn spray only, when leaf-fall was imminent.
S—Spring spray only, just before the blossoms opened.
AS—The above two treatments in sequence.

The thirteen different rootstocks involved were distributed as uniformly as possible among the treatments; indeed, differing rootstocks, complicating the lay-out of the trial, formed the basis of its plan.

The trial began in autumn, 1934, but the spray could not be put on before November 27th, when leaf-fall was almost complete. The spring spray followed on April 12th, 1935, when the flower-buds were still in fairly tight cluster, and several days before the first flowers opened. A $2\frac{1}{2}$ h.p. portable machine, delivering a fairly straight, driving spray at 200-250 lb. pressure through a double nozzle with $\frac{4}{64}$ in. disc-orifices, gave satisfactory coverage.

The number of cankers was recorded, and affected branches and lateral shoots were cut out in summer. Leaf-Spot was assessed at the same time, each tree being allotted, by inspection, a category in the range 0 (nil or a trace) to 3 (severe Leaf-Spot).

This procedure was adopted each year; any noteworthy deviations will be mentioned below.

It will be seen that spraying only slightly reduced the incidence of cankers in this first season. The spring spray reduced Leaf-Spot.

* *Prunus avium* L.—the sweet cherry (Gean, Gaskin, or Mazzard).
† 10 lb. powdered copper sulphate crystals and 15 lb. hydrated lime per 100 gallons of wash, made in the spray-tank by adding strong copper sulphate solution to weak milk of lime.

TABLE I.

Cankers and Leaf-Spot recorded July, 1935. Averages per tree.

Treatment.	No. of Trees.	No. of New Cankers.	Leaf-Spot Category.
A	20	2·1	1·2
S	20	2·4	0·5
AS	20	2·2	0·7
C	9	2·9	1·0

Second Year, 1935-36. The trial was elaborated to include the following comparisons:

(i) One autumn spray (**A**), just before leaf-fall as hitherto, with two (**AA**), one just before and the other during leaf-fall.

(ii) One spring spray (**S**), just before blossom as hitherto, with two (**SS**), one just before and the other just after blossom.

There were also certain combinations of these. A spray applied just before leaf-fall would dry around the leaf-bases and in the leaf-axils, and later protect the leaf-scars during rain or dew, when infection is likely. The second autumn spray, and the second spring spray for the leaves, would renew the protective coverings.

The sprayed trees were now in units of ten instead of twenty and, using the symbols as before, the treatments were: **A**, **AA**, **S**, **SS**, **ASS**, **AAS**, **C**. Continuity with the first year's trial was maintained by choosing ten of the trees hitherto once-sprayed in autumn or spring for a second spray at either period.

The applications were:

1st autumn, September 26th, 1935. Leaf-fall not yet begun.

2nd autumn, November 12th, 1935. Unsprayed controls and spring-sprayed trees had lost about one-half of their leaves, but those sprayed on September 26th were nearly bare, the Bordeaux having caused early defoliation.

1st spring, April 14th, 1936. A few days before the first blossoms opened.

2nd spring, May 18th, 1936. Blossoms just over.

TABLE II.

Cankers and Leaf-Spot, July, 1936. Averages per tree.

Treatment.	No. of trees.	New cankers. As small gum centres around buds.	New cankers. On branches, etc.	Summary.	Leaf-Spot category.
A	10	2·7	5·9	**A** 7·8	0·6
AA	9*	2·0	5·0		0·5
S	10	6·3	3·8	**S** 9·9	0·6
SS	10	7·0	2·6		—†
ASS	10	2·8	2·9	**AS** 5·7	—†
AAS	10	2·5	3·2		0·3
C	8*	9·2	8·0	**C** 17·2	1·0

* One tree died. † Not recorded (see text).

The second spring spray caused severe leaf-spotting. The small, isolated, scorched, irregularly angular areas ultimately gave the leaves a "shot-hole" appearance, at first glance very like Bacterial Shot-Hole, in which, however, the spots are nearly circular. It was, therefore, impossible to assess bacterial infection of the foliage correctly. These damaged leaves developed autumn colouration and dropped prematurely, the affected trees being half-bare by mid-October (1936) when the others were still very leafy.

Autumn spraying checked the incidence of small bud-infections, and spring spraying reduced the number of branch cankers. Two applications showed no marked improvement over one at either period, though there were fewer branch cankers with two spring sprays than with one, where no autumn spray was given.

When all infections are considered together, autumn spraying and spring spraying each reduced infection by about one-half, as compared with controls. Combined autumn and spring spraying gave the best control, reducing infection by about two-thirds. There was only slight Leaf-Spot, even on control trees, although there was very severe cankering.

Third Year, 1936-37. A further modification was made in the combined autumn and spring treatments, the comparison **AS** with **AASS** being included instead of **ASS** with **AAS**. Thus two applications were compared with one at both periods as well as at each.

The applications were:

1st autumn, November 13th, 1936. Defoliation very variable, up to about 75 per cent. Trees sprayed twice in spring virtually bare.
2nd autumn, December 3rd, 1936. Trees defoliated.
1st spring, April 19th, 1937. First flowers opening.
2nd spring, May 13th, 1937. Blossoms just over.

A new 2 h.p. mobile spraying machine developing 300 lb. pressure was used for the spring sprays, and the higher pressure greatly facilitated the work. Bordeaux mixture was used at 6-9-100 instead of 8-12-100 for both of the spring sprays because of the damage caused in 1936 by the stronger spray. This reduction was not enough, however, for severe yellowing and spotting of leaves resulted, mainly from the second application made when there was much more foliage to be damaged. The fruits were not damaged.

TABLE III.

Cankers, September, 1937. Averages per tree.

Treatment.	No. of trees.	New cankers.		Summary.
		As small gum centres around buds.	On branches, etc.	
A	9*	0·9	3·1	**A** 3·9
AA	9	1·1	2·7	
S	10	1·3	2·1	**S** 2·7
SS	10	1·1	0·9	
AS	10	0·3	0·4	**AS** 1·2
AASS	10	0·8	0·9	
C	8	1·4	8·3	**C** 9·7

* One tree died.

A general survey of the trees on October 21st, 1936, revealed fresh cankers, which must have developed since the cutting-out in July, and suggests that branch cankers might be initiated in a wet summer like that of 1936, though this is unusual. Wormald (*loc. cit.*), who found that the bacteria in cankers usually die out in summer, also mentioned the unusual circumstances of 1936, when living bacteria were found in cankers in July and August. The inoculum for these fresh cankers, from which trees sprayed twice in spring were practically free, may have been bacteria from leaf-spots, or from earlier cankers before their removal. Leaf-Spot was slight in July, however, as the data show. Montgomery *et al.* (1943) noted the presence of fresh branch cankers on Victoria plum in autumn, 1941; an unusual development that itself suggests the possibility of branch-infection during the growing season. Foliage spraying with Bordeaux provided control also in this instance.

There was only a trace of Leaf-Spot in 1937, and cankers were much less prevalent than in the previous year. Some of those recorded in Table III were known to have been present in the previous autumn. Two applications again showed no marked improvement over one, though once more a second spring spray further reduced the number of branch cankers in the absence of autumn application. Spring spraying and autumn spraying separately gave marked control, but when combined in sequence they reduced the disease to very small proportions as compared with that on the controls. Doubtless the cumulative effect of three seasons' spraying was now becoming apparent.

Fourth Year, 1937-38. Mid-October was found to be a good time for the first autumn spray because leaf-fall normally occurs between then and mid-November. Hence, the applications were:

1st autumn, October 12th, 1937. No leaf-fall except where Bordeaux mixture was put on in May. Here the trees were about half-defoliated.

2nd autumn, November 11th, 1937. Leaf-fall very rapid during the previous week. Some trees, especially unsprayed controls, were still fairly leafy, but this was very variable.

1st spring, March 29th, 1938. A few days before the first blossoms opened; a very early season.

2nd spring, May 4th, 1938. Blossoms just over.

Bordeaux mixture was again used at 6-9-100 for the first spring spray, but for the second it was reduced to 4-6-100. Edible cottonseed oil (0·75 per cent. by volume) was incorporated with

TABLE IV.

Cankers and Leaf-Spot, August, 1938. Averages per tree.

Treatment.	No. of trees.	New cankers. As small gum centres around buds	New cankers. On branches, etc.	Summary.	Leaf-Spot.
A	9	0·8	1·3	A 1·6	1·8
AA	9	0·7	0·3		1·6
S	10	0·9	1·3	S 1·4	1·2
SS	10	0·1	0·5		0·2
AS	10	0·2	0·3	AS 0·6	0·9
AASS	10	0·1	0·6		trace
C	8	2·1	2·4	C 4·5	1·8

the second spray to minimize spray damage. Sprayed foliage remained excellent throughout the season, no leaf-spotting and only very slight purpling of the leaf-margins being seen at any time. A similar result was obtained on Victoria plum (Montgomery *et al.*, 1943), and, earlier, on apple (Moore, 1938).

Leaf-Spot was severe on autumn-sprayed trees and controls; it was slightly checked by the pre-blossom spray, and virtually eliminated by the petal-fall one. There was only slight cankering on sprayed trees, but much more though not excessive on the controls. Two sprays were more effective than one, especially in spring, except where autumn and spring sprays were combined in sequence. Spring sprays and autumn sprays separately reduced the number of cankers and bud-infections together by two-thirds, but when in sequence they gave almost complete control.

A subsidiary trial was carried out on some other Schrecken trees on the same plot to determine whether any damage to the flowers would accrue from spraying trees when in bloom. The trial was inconclusive owing to severe frost which damaged the blossoms. It showed, however, that Bordeaux (6-9-100) with cottonseed oil (0·75 per cent.) caused very much less leaf-damage than the same spray without oil (Plate I).

Fifth Year, 1938-39. While the sprayed Schrecken trees had by this time been restored to very good commercial condition, the Rivers had gradually deteriorated, and were severely cankered. It was decided, therefore, to apply the spray-programme (**AS**) to all three varieties, though the Turkey Heart trees had never had more than an occasional infection. In addition, the Schrecken trees in one half of the plot received a second spray at each period (**AASS**) to continue the comparison with only one (**AS**) in the other half.

Records made on Schrecken in July, 1939, showed that there was practically no Canker or Leaf-Spot on either half-plot, including the old controls, so that the disease had been virtually eliminated from these susceptible trees. With the onset of war the comparison had to be abandoned, but the routine was continued.

The influence of seasonal conditions on bacterial canker.

Only one recording of Canker and Leaf-Spot was made each year so that a detailed comparison of their incidence (particularly of Leaf-Spot) with rainfall is not possible. Low incidence of Canker in 1935 followed rainfall less than average in every month from August to November, 1934 (total 7·23 in.). Very severe Canker in 1936 followed the wet autumn of 1935, when rainfall was exceptionally high from August to November (total 14·65 in.), especially in September (3·57 in.) and November (5·15 in.). June, 1936, also had 3·95 in., more than 2 in. above average, and this may have been partly responsible for the development, possibly following earlier infection, of fresh cankers after recording had been completed in July, 1936.

The influence of rootstock on bacterial canker and leaf-spot (*var. Schrecken*).

This can be seen in Table V, where the data are classified according to rootstock and regardless of spray treatment.

The data merely give an indication of the *relative* susceptibility of Schrecken on the various rootstocks because of the variable manner in which differential spray treatments had to be superimposed. For instance, of twelve trees on F12/1, tentatively classed as relatively resistant, two were regularly sprayed in autumn only, four in autumn and spring combined, and five in spring only, while only one was unsprayed. Trees on F5/2 provide a similar instance. By contrast, of five trees each on F2/1, F5/3, and F5/4, tentatively classed as susceptible, four were regularly sprayed at one or both periods and one was unsprayed, so that a bias in favour of the relative resistance of F5/2 and F12/1 is introduced. Nevertheless, at least two examples provide an argument the other way; trees on F12/4 showed consistent susceptibility and seven out of the eight were regularly sprayed, while those on F5/5 showed relative resistance and one of the four

TABLE V.

Influence of rootstock on susceptibility to Bacterial Canker and Leaf-Spot in Schrecken (I). Averages per tree.

Rootstock. Schrecken on :	No. of trees.	Cankers. Year : 1935.	1936.	1937.	1938.	Av.	Leaf-Spot. Year : 1935.	1936.	1937.†	1938.	Av.	Remarks.
F 1/1	7	0·6	7·9	2·9	1·1	3·1	0·4	0·4	—	1·4	0·7	relatively resistant
2/1	5	5·2	16·2	4·0	2·2	**6·9**	0·8	1·1	—	1·1	**1·0**	**susceptible**
*2/2	3	2·0	8·7	6·7	1·3	4·7	0·7	0·6	—	1·3	0·9	moderately susceptible
*5/1	2	4·5	3·5	0·5	2·0	2·6	0·6	0·5	—	2·0	1·0	inconclusive
5/2	10	1·4	6·1	3·6	0·8	3·0	0·8	0·4	—	1·1	0·8	relatively resistant
5/3	5	6·0	11·0	6·2	2·0	**6·3**	1·5	0·6	—	1·7	**1·3**	**susceptible**
5/4	5	2·2	10·8	7·2	2·6	**5·7**	1·3	0·6	—	1·2	**1·0**	**susceptible**
5/5	4	2·0	8·0	2·0	0·5	3·1	1·0	0·4	—	1·0	0·8	relatively resistant
9/1	4	3·5	11·0	1·3	1·0	4·2	1·0	0·1	—	0·5	0·5	moderately susceptible
12/1	12	2·4	6·7	2·8	1·3	3·3	0·7	0·6	—	0·2	0·5	relatively resistant
*12/3	2	2·0	2·5	5·5	3·0	3·3	0·4	0·0	—	trace	0·1	inconclusive
12/4	8	3·3	11·8	5·4	2·0	**5·6**	0·7	0·7	—	1·3	**0·9**	**susceptible**
*12/5	2	1·5	8·0	2·3	7·0	3·2	0·6	0·5	—	0·5	0·5	inconclusive
Yearly average:		2·8	8·6	3·9	1·6		0·8	0·5	trace	1·2		

* These stocks were not included among unsprayed control trees.
† Only a trace of Leaf-Spot present.

was unsprayed. The annual record of the nine individual *unsprayed* trees (Table VI) provides general support for the data above.

TABLE VI.

Influence of rootstock on susceptibility to Bacterial Canker in Schrecken (II).

Rootstock. Schrecken on :	Severity of the disease in : 1932.	1933.	1934.	1935.	1936.	1937.	1938.	Seven-year average.	Remarks.
F 1/1	1	1	3	1	1	1	1	1·3	R
2/1	2	1	2	1	4	2	1	**1·9**	**S**
5/2	1	1	2	1	3	3	1	1·7	S
5/3	2	1	3	2	3	3	1	**2·1**	**S**
5/4	1	1	3	1	3	4	3	**2·3**	**S**
5/5	1	1	1	1	2	1	1	1·1	R
9/1*	1	1	3	3	dead	—	—	2·0†	S
12/1	2	1	2	1	1	1	2	1·4	R
12/4	2	1	3	1	3	3	2	**2·1**	**S**

* Small tree, replant. † Four-year average.

Key : 1, up to 5 cankers ; 2, 6 to 10 cankers ; 3, 11 to 15 cankers ; 4, more than 15 cankers.
R=relatively resistant. S=susceptible.

It will be seen that the four rootstocks—F2/1, 5/3, 5/4, 12/4—classed as inducing susceptibility in sprayed and unsprayed trees together over a four-year period, induced also the most clearly-defined susceptibility on unsprayed trees alone. Similarly, the three rootstocks F1/1, 5/5, and 12/1 are classed in both series as inducing relative resistance. F5/1, 12/3, and 12/5 gave inconclusive results because there were very few trees on each rootstock, with no controls; in the sprayed series they remained relatively resistant, perhaps because of the spray treatment. The trees on F2/2 can, however, be regarded as moderately susceptible because, in spite of the absence of unsprayed trees among them, they became fairly severely cankered. Those on F9/1 appear to be at least moderately susceptible when the two series of data are considered together. The only real anomaly is provided by trees on F5/2; the unsprayed tree showed fairly high susceptibility over the seven-year period, whereas the ten trees on this stock, including the one control, showed relative resistance during the trial. The individual tree records suggest that trees on this stock will show relative resistance rather than marked susceptibility, but that resistance may be modified in certain seasons, especially when the trees are not sprayed.

The influence of rootstock on spray damage (*vars. Napoleon, Early Rivers, and Frogmore*).

This was shown by tests on two different randomized plots of trees undergoing rootstock trials.

I. Trees of Napoleon, worked high or low on each of four selected series of seedling Mazzard rootstocks (F230, 231, 232, and 260) and one layered Mazzard (F12/1), were sprayed with Bordeaux mixture (8-12-100) in May, 1936, and some were not sprayed. The spray caused severe leaf-scorch and defoliation, and category records (max. 3·0) on sprayed trees gave the following mean results:

Table VII.

Influence of rootstock on spray damage in Napoleon.

	F230.	F231.	F232.	F260.	F12/1.
High-worked stock ..	2·1	1·9	3·0	1·3	1·7
High-worked scion ..	0·7	1·2	1·0	0·5	1·8
Low-worked scion ..	1·3	1·1	1·0	0·4	1·3
Average for scion ..	1·0	1·2	1·0	0·5	1·6

The F260 series, itself more resistant than the others, imparted some resistance to Napoleon, which formed vigorous, healthy trees on them. On F12/1 the variety was less vigorous and more damaged by the spray. The rootstock leaves of F232 were very severely affected.

II. Trees of Early Rivers and Frogmore, low-worked on each of the Mazzard rootstocks F2/1 and F5/4 and on the much less vigorous acid cherry rootstock F4/1, were sprayed at the same time as the Napoleons, and similar damage resulted on Rivers, while on Frogmore there was very little. Unsprayed trees of both varieties showed no damage. Category records (max. 3·0) on the sprayed trees gave the following mean results:

Table VIII.

Influence of rootstock on spray damage in Early Rivers and Frogmore.

	F2/1.	F4/1.	F5/4.
Rivers	3·0	2·1	3·0
Frogmore	1·0	0·4	0·9

The acid cherry stock F4/1 induced relative resistance in both varieties, which themselves greatly differed in susceptibility.

The remaining leaves of all sprayed trees of Napoleon, Rivers, and Frogmore* reddened and dropped early in autumn, as with Schrecken.

DISCUSSION AND RECOMMENDATIONS.

Four seasons' spraying of trees of the very susceptible Bigarreau de Schrecken, severely infected when the treatment began, sufficed to restore them to sound commercial condition. Two sprayings every season were necessary, one during the second half of October, just before leaf-fall, and the other in April, just before the blossoms opened. Each gave partial control, but the two in sequence had almost eliminated infection by the end of the period. Success cannot be expected at the end of only one season's treatment ; *both* sprays should be put on at the correct periods over several seasons. There was some evidence that a second spraying in both autumn and spring, and especially one in spring, still further reduced infection in certain seasons, but the evidence was complicated by the presence of a differential rootstock factor, and is therefore not conclusive. The spray used was Bordeaux mixture, at 10-15-100 in autumn and at 6-9-100 pre-blossom ; the use of 8-12-100 in spring was abandoned as unsafe. A petal-fall spray at 8-12-100 or 6-9-100 was followed by early autumn colouration and defoliation. In another experiment, on Victoria plum, trees sprayed with Bordeaux (4-6-100) in early summer retained their foliage longer in autumn than did unsprayed trees or those sprayed at or before petal-fall (Montgomery *et al.*, 1943), though the issue was complicated in this case by the presence of Plum Rust (*Puccinia Pruni-spinosae*), which itself caused defoliation and was partially controlled by the copper sprays. For post-blossom use 4-6-100 was as strong as the trees would tolerate, and even this would probably cause leaf-spotting when followed by wet weather. Excellent foliage resulted where cottonseed oil (0·75 per cent. by volume) was incorporated with Bordeaux (4-6-100) in 1938. A new trial is in progress to compare certain other sprays with Bordeaux mixture.

It is of interest that this programme of sprays was highly successful on Schrecken, and yet a similar, though not identical, programme yielded inconclusive results on Victoria plum (Montgomery *et al.*, 1943). There is, however, no reason to suppose that the two-application programme for Schrecken would not be successful for other susceptible sweet cherry varieties such as Napoleon, Florence, Bradbourne Black, Early Rivers, and Ohio Beauty. In a mixed orchard with Rivers present, the spring spray should be put on all susceptible varieties when the Rivers's blossoms are about to open.

High autumn rainfall appeared to favour the incidence of Canker in the following year.

The data obtained indicated that certain Mazzard rootstocks varied in their capacity for inducing susceptibility to Bacterial Canker and Leaf-Spot in Schrecken, F2/1, 5/3, 5/4, 12/4, and possibly 9/1, tending towards high susceptibility, and F1/1, 5/5, 12/1 and possibly 5/2, to relative resistance, with F2/2 intermediate. This classification is tentative for reasons already given, and the few trees respectively on F5/1, 12/3, and 12/5 are not included. An influence of rootstock on liability to spray damage to foliage on Napoleon, Rivers, and Frogmore was shown by a subsidiary trial.

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* Many Frogmore buds had been killed by Bacterial Canker, which did not progress into the branches. Napoleon, however, was severely cankered and showed profuse gumming.

PLATE I.

Bigarreau de Schrecken cherry shoots; on left, sprayed at petal-fall with Bordeaux mixture (6-9-100), and on right, with Bordeaux mixture (6-9-100) plus edible cottonseed oil (0·75 per cent. by volume). Note Bordeaux damage to leaves in left-hand specimen, absent in that on right.

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SUMMARY.

Bacterial Canker, due to *Pseudomonas mors-prunorum* Wormald, was well controlled over four seasons on the susceptible Bigarreau de Schrecken cherry by spraying with Bordeaux mixture both in autumn (at 10-15-100) and spring (at 6-9-100 pre-blossom). Each spraying separately contributed partial control, while spring sprays, especially that at petal-fall, controlled moderate Leaf-Spot. Foliage damage followed the use of this spray stronger than 4-6-100 post-blossom, but the addition of edible cottonseed oil (0·75 per cent. by volume) prevented this in the one season in which it was tried. The incidence of the disease on Schrecken was influenced by rootstock and by seasonal conditions, and that of spray damage on Napoleon, Rivers, and Frogmore by rootstock.

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THE USE OF DINITROCRESOL-MINERAL OIL SPRAYS FOR THE CONTROL OF PROLONGED REST IN APPLE ORCHARDS

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REST, as a factor affecting the time of opening of buds, was studied by Jost (12) as early as 1893, and since then investigations have been continued, mostly under greenhouse conditions. However, its importance as a major problem of fruit growing was not recognized until recently, when Weldon (24), in California, and Reinecke (16), in South Africa, showed that prolonged rest, due to insufficient winter-chilling, affects fruit trees under field conditions to such an extent that in sub-tropical countries it becomes one of the limiting factors of deciduous fruit culture.

In the course of a survey of deciduous fruit orchards in Palestine by the author (unpublished), symptoms of prolonged rest, such as delayed bloom and foliation (15), irregular and protracted period of buds opening (7), failure of a large number of buds to open at all (6), as well as bud abscission (16), were noticed for several years in all parts of the country. These field observations were checked against mean winter temperatures, using the method developed by Weldon (24), in California, and verified by De Villiers (21) under South African conditions. It was found (17) in those lowlands of Palestine where apples are being grown, that winter temperatures in each of the past ten years were above those required for normal termination of the rest-period (average mean temperatures for the two winter months December and January ranged for the years 1930-1940 between 9·9-16·5° C.), while at higher elevations, such as Jerusalem (about 800 m.; 2,600 ft.), only three of those ten years were cool enough to be conducive to normal growth.

This situation led to research designed towards the control of this factor by artificial means, in addition to variety selection and breeding. Different methods of breaking rest are known, but spraying seemed the most promising under commercial conditions. Although various entomologists, such as Essig (cited by Burroughs (2)) and others, in the United States, and Mally (14), in South Africa, observed early that mineral oils hasten blossoming, the first systematic study of the effect of oils from the horticultural standpoint (apart from shorter reports (2,8)) was undertaken by Black (1) who used the proprietary mineral oil emulsions "Kleenup" and "Pestridol". Chandler and his co-workers (4) reported extensive observations concerning the effect of a 2, 4-dinitro-6-*cyclo*hexylphenol (DNO)-mineral oil spray on a large variety of plant material. This stimulated a trial, on peach twigs, of twenty-five different cyclic compounds and their dinitro derivatives by Weinberger (23) who found that dinitrophenol had marked rest-breaking properties. Guthrie (10), who tested forty cyclic dinitro- and thio-compounds, reached the conclusion that *p*-thiocresol, 4-chloro-*o*-phenylphenol and α-nitro-naphthalene break the rest of peach buds. In our own trials, which are still in progress, it became apparent in 1939 that 3,5-dinitro-*o*-cresol (DNC) aids the action of mineral oil in breaking rest similarly to DNO. DNC was introduced into the entomological literature on spray materials by Tattersfield *et al.* in 1925 (20), and Shaw and Steer (18) found it to be the most toxic to insect eggs of all the forty-four organic compounds which they tried. The great similarity in rest-breaking properties between DNC and DNO, as compared with the numerous other materials tried by the above investigators, might be more easily understood, if DNC is viewed (13) as 2,4-dinitro-6-methyl phenol, i.e. as the simplest member of the series 2,4-dinitro-6-R-phenol to which DNO belongs. The results obtained in the orchard by the use of DNC-mineral oil applications in breaking the rest of apple trees form the subject of this paper.

MATERIALS AND METHODS.

The stock spray material consisted of a medium heavy mineral oil with a 70-75 per cent. unsulphonated residue. In the oil about 1·5 per cent. DNC was dissolved.* Four parts of this material were emulsified with one part of water and emulsifier, and 5 per cent. of this emulsion was used to make up the spray liquid which thus contained 4 per cent. of actual oil and 0·06 per cent. DNC. The spray was applied in all cases, when not stated otherwise, by means of a power sprayer with about 20 atmospheres pressure.

The effect of the treatment was recorded by observing its action on two symptoms of prolonged rest : (*a*) the delay in the opening of buds, and (*b*) the non-awakening of buds. Treated shoots were left unpruned in order to avoid the traumatic effect of wounds. The amount of delay of foliation and bloom was recorded in the first experiments by comparing the dates when the buds had reached a certain stage of development. Counts of all blossom and leaf buds on the entire tree were used as basis for these determinations. Thus, the date of 50 per cent. bloom was determined as follows : when the tree was judged to have opened about 30-50 per cent. of its blossoms, all open blossoms were counted (*a*). At a later date, when 50-80 per cent. of the blossoms were judged to have opened, counts were made again of all open blossoms together with those which had already shed their petals (*b*) ; and swollen blossom buds which had not as yet opened were counted separately (*c*). (*b*) plus (*c*) was the basis (total blossom buds) for the calculation of actual percentages of buds which had been found open at both dates. The date of 50 per cent. bloom was then found by interpolation between the two dates.

Because these counts were rather laborious, phenological observations were recorded and compared with those obtained by count. While phenologists dealing with regions with cold winters have laid great weight on the date of opening of the first blossom, such procedure is not quite adapted to conditions under which prolonged rest prevails, because beginning of bloom straggles on for some time. Hence more appropriate standards had to be chosen. " Beginning of bloom " under these conditions was therefore defined as the date when the first few blossoms opened and the remainder of the buds had swollen sufficiently to indicate the approach of the main blossoming period. Although this definition may appear rather vague, still it describes a quite definite stage of development in the orchard. Similarly, " end of bloom " had to be defined as the stage when the main blossoming period was over and but a few blossoms were left on the tree. A third stage, which is the most easily recognized, is that of full bloom, when the first blossoms begin to shed their petals. In establishing the validity of phenological standards a comparison of the dates of 50 per cent. bloom, as determined by actual counts, with those of observed full bloom and those of " mid-bloom " were made. For " mid-bloom " the average between the mean of dates of beginning and end of bloom and the date of full bloom was taken, thereby giving greater weight to the latter. A comparison of the data thus obtained is given in Table I.

The close agreement between the dates arrived at by the three methods is apparent. Mid-bloom was chosen as preferable to full bloom, because this date is based on three observations, thus reducing errors which have to be reckoned with when using observational data rather than counts.

In a similar way mid-foliation was found both by counts and observations. Date of mid-foliation is less reliable than the date of mid-bloom, because foliation, particularly on the more vigorous shoots, continues for some time into the summer, and the readiness of leaf buds to open is more difficult to recognize than that of blossom buds. Date of mid-foliation was calculated by averaging the dates of beginning of foliation (i.e. when growth from leaf buds reached the dimensions of a small brush 1-2 cm. in length) and full foliation, when the first leaf on the first few shoots spread open and reached full size.

* This concentration is close to the limit of solubility ; part of the nitro-cresol in the emulsion goes over from the oil to the water phase.

TABLE I.

Comparative results of methods of recording blossoming data.
(All numbers refer to dates in April, 1939.)

Name of Variety.	Rootstock.	Date of bloom by Observation.		Count.
		Full bloom.	Mid-bloom.	50% bloom.
Banana	Khashabi*	15	14	14
,,	Seedling	15	14	17
Red Astrachan	Doucin	23	21	23
,, ,,	Khashabi	24	24	25
San Sauveur	Doucin	21	20	20
Queen Pippin	,,	25	27	27
Peasgood	,,	25	27	27

* Local semi-vigorous stock.

Harvest dates were recorded by noting the number of fruits of a definite state of maturity (colour) which were picked every other day. Date of 50 per cent. harvest could thus easily be determined. Spread of " main harvest " denotes the number of days between the date at which 25 per cent. of the fruits were harvested and that at which 75 per cent. had been taken off the tree. This was found useful because of the straggling of fruit maturation at both ends of the season.

Although primary attention was originally given to delay of foliation and blossoming, the fact that, under conditions conducive to delayed foliation some of the buds do not open at all and remain dormant throughout the season, is perhaps even more important from the horticultural point of view. In the first experiments all open and closed buds on the entire tree were counted ; but, in addition to the difficulty of counting such very large numbers, such a procedure rather increases than decreases variability. Chandler and Tufts (5) have shown that more vigorous shoots are more affected by prolonged rest than shorter growths, even on the same tree. Relative vigour of trees, as expressed by the number of shoots of different lengths as well as by the development of fruit wood, would then be expected to affect greatly the relative number of buds remaining dormant on the tree as a whole. By actual test the opening of buds on representative one-year-old shoots was found to be a more accurate as well as a more convenient measure of response to treatment.

In order to standardize the choice of shoots, buds were counted on shoots of different lengths on two trees of each of seven treatments of Queen Pippin (Reine des Reinettes) and of four treatments of Delicious. Buds on the lower and the distal parts of the branches less than 1 cm. distance from each other were not considered, thus eliminating part of the less developed basal and sub-terminal buds. Correlation diagrams showed that there was no correlation between percentage of buds that open and length of shoot between 50 cm. and 2 m., but that frequently a considerably higher percentage of buds opened on shorter shoots below 50 cm. in length. Since Reinecke (16) had found that the north-side of deciduous trees in the southern hemisphere was more injured by prolonged rest, counts were made separately on shoots according to the geographical quadrants of the trees, in order to test the effects of differences in insolation. No consistent differences between any two quadrants could be found with our material, as measured by percentages of buds opening. Apparently the amount of shading by the defoliated branches was immaterial. Representative number of shoots was found by determining the variability of random groups of different size, and twenty-five shoots were found to form an adequate representative sample. Thus, the counting of buds was carried

out on twenty-five one-year-old shoots, at least 50 cm. long, distributed at a convenient height all around the crown.

The significance of the data was tested according to Fisher's table for "t" (9), following the procedure outlined by Hoblyn (11) using single tree plots. As will be seen, there was considerable variability which differed with varieties and localities, and even with the same variety and locality in different years. Since six repeats still showed considerable fluctuations, the use of grown-up trees at wide planting distances was finally abandoned, and trees planted closely together (1 × 2 m.) and pruned short in order to obtain vigorous shoots are now used. The spray material is applied by means of a brush rather than by a spraying machine, and it is expected that more uniform material will be obtained in this way. The experiment, Rehovot, 1943 (Table V), was carried out on this material while all other experiments were made on young bearing trees according to the method described above.

EXPERIMENTAL.

Time of awakening.—As may be seen from Fig. 1 (Plate I) spraying with DNC-mineral oil emulsion considerably affects the awakening in spring of trees suffering from prolonged rest.

Similar results were obtained with a number of varieties although in varying degree. However, it was observed that date of spraying seemed to affect earliness of awakening. Therefore fourteen different apple varieties were sprayed at approximately weekly intervals between the beginning of February and the end of March. The data concerning the influence of those treatments on date of mid-bloom are presented in Table II.

TABLE II.

The effect of date of spraying on date of mid-bloom. Mishmar Haemek (Jesreel Valley).

	Date of mid-bloom, April, 1940.													
Date of spraying.	Banana.	Red Astrachan.	Hammerstein.	Calville San Sauveur.	Delicious.	Croncelles.	White Calville.	Queen Pippin.	Jacob Lebel.	Rome.	Gravenstein.	Peasgood.	Jean Sucree.	Boscoop.
February 8	29.III.	1	1	4	4	7	9	11	12	11	14	19	25	20
15	6.IV.	6	6	11	9	7	10	12	16	12	16	18	23	20
23	10	15	15	14	19	25	18	18	17	20	23	22	—	—
29	11	10	11	14	13	20	18	18	19	17	17	21	24	19
March 7	14	15	15	16	18	—	19	19	21	20	20	22	24	23
13	16	19	18	19	19	—	21	20	21	21	24	25	24	25
25	16	20	21	23	24	23	(23)	25	23	25	24	26	25	24
Unsprayed control	16	21	22	22	25	25	25	(26)	25	26	26	28	27	29
Number of days date of mid-bloom is advanced by earliest treatment	18	20	21	18	21	18	16	15	13	15	12	9	2	9

The data for all varieties show clearly that the earliest treatment had the maximum influence on advancing time of blossoming and that the later the spraying, the later the bloom. While spraying eleven weeks before normal mid-bloom advanced the blossoming of some varieties by as much as three weeks, spraying as late as four weeks before mid-bloom had little if any effect on time of blossoming. This observation has been made repeatedly in later years and at different places, with only minor fluctuations in the very regular trend. The outstanding exception of the treatment of February 23rd is probably due to the fact that spraying at that

date was carried out by means of a knapsack sprayer, because, two days before, heavy rains had softened the ground to such an extent, that it was impossible to enter the orchard with a power sprayer. In view of the fact that the deviations which reduce the effectiveness of the treatment on this date were found to be particularly erratic when based on observations concerning the entire tree, while bud counts on branches at a convenient height for spraying showed greater normality, it is likely that spray coverage on that day was incomplete.

The varieties are arranged in the Table in the order of time of blossoming. It will be noticed that the sequence of bloom of different varieties is generally not affected by the treatments. The effectiveness of the spray, however, as expressed by the number of days of advance of bloom over that of the control differs considerably between varieties. This is brought out clearly in the bottom row of the Table where are to be found the number of days of advance of blossoming, obtained by the most effective treatment, on February 8th. It ranges from three weeks with Hammerstein to two to five days with Jean Sucree. It would seem that the effectiveness of the spray treatment on advance of bloom of different varieties decreases with individual property of later natural bloom. Therefore the spread between bloom of different varieties will be increased by early spraying. In this case it will be found that, e.g. twenty-two days elapsed between the bloom of Banana and Boscoop if sprayed on February 8th, as against thirteen days for the unsprayed control.

Dates of foliation are, as would be expected, similarly affected by spraying at different times and show a trend similar to that of dates of bloom. Still there is a slight consistent difference of response as shown in Table III, in which dates of mid-bloom are compared with dates of mid-foliation.

Table III.

The effect of spraying on the interval between time of bloom and foliation. Mishmar Haemek, 1940.
(The negative sign indicates that mid-foliation took place before mid-bloom.)

Name of variety.	Unsprayed control.			Sprayed.					
				February 8th.			March 25th.		
	Date of			Date of			Date of		
	Mid-bloom.	Mid-foliation.	Days difference.	Mid-bloom.	Mid-foliation.	Days difference.	Mid-bloom.	Mid-foliation.	Days difference.
Banana ..	16.IV.	19.IV.	3	29.III.	4.IV.	6	16.IV.	17.IV.	1
R. Astrachan	21.IV.	23.IV.	2	1.IV.	11.IV.	10	20.IV.	20.IV.	0
Hammerstein	22.IV.	27.IV.	5	1.IV.	10.IV.	9	21.IV.	21.IV.	0
San Sauveur	22.IV.	24.IV	2	4.IV.	8.IV.	4	23.IV.	23.IV.	0
Delicious ..	25.IV.	3.V.	8	4.IV.	23.IV.	19	24.IV.	23.IV.	−1
Croncelles ..	25.IV.	3.V.	8	7.IV.	20.IV.	13	(23.IV.)	24.IV.	1
W. Calville ..	25.IV.	28.IV.	3	9.IV.	16.IV.	7	23.IV.	18.IV.	−5
Queen P. ..	(26.IV.)	2.V.	6	11.IV.	18.IV.	7	25.IV.	23.IV.	−2
J. Lebel ..	25.IV.	3.V.	8	12.IV.	16.IV.	4	23.IV.	23.IV.	0
Rome ..	26.IV.	29.IV.	3	11.IV.	20.IV.	9	25.IV.	23.IV.	−2
Gravenstein	26.IV.	29.IV.	3	14.IV.	16.IV.	2	24.IV.	26.IV.	2
Peasgood ..	28.IV.	1.V.	3	19.IV.	21.IV.	2	26.IV.	25.IV.	−1
Jean Sucree	27.IV.	27.IV.	0	25.IV.	24.IV.	−1	25.IV.	26.IV.	1
Boscoop ..	29.IV.	3.V.	4	20.IV.	22.IV.	2	24.IV.	22.IV.	−2

In spite of considerable fluctuations, due primarily to the difficulty of observing stages of foliation, the following facts would seem to emerge from these data: Firstly, under conditions of prolonged rest (unsprayed control) mid-bloom of apples would seem generally to take place before mid-foliation. This is also the case with trees sprayed early (February 8). As a matter

of fact, with most varieties, this tendency would seem to be even more accentuated by early spraying, although with a few others the time-lapse between bloom and foliation was reduced with those trees as compared with the control. Further study is required to verify the significance of these variations. What seems, however, most interesting is the consistent evidence that late spray application affected leaf buds somewhat more than blossom buds, so that foliation coincided with bloom, and in some cases even preceded it.

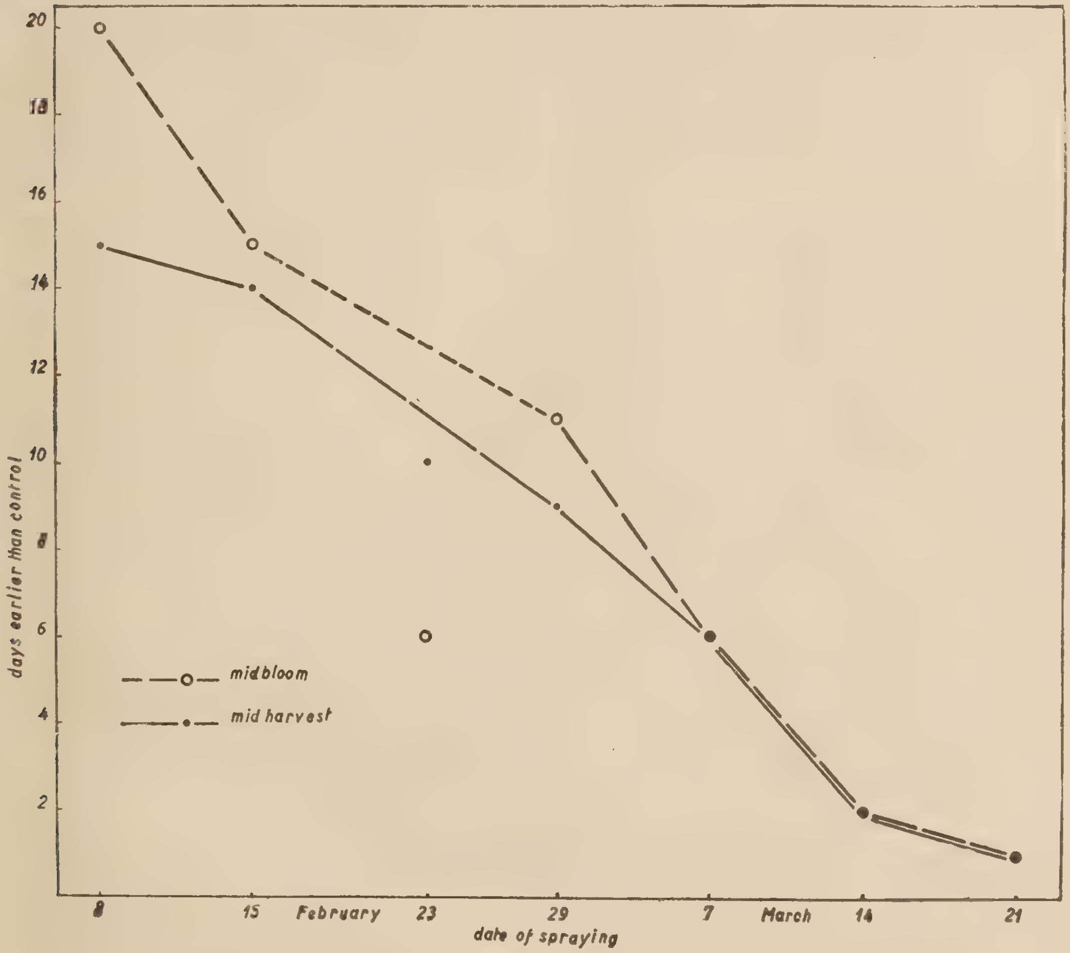

FIG. 2.

Effect of time of spraying on advance of bloom and maturation of Red Astrachan. Mishmar Haemek, 1940.

Earlier bloom would also be expected to cause earlier maturity. This is shown to be so with Red Astrachan (in which the stage of maturity is easily recognized by colour development) in Fig. 2 and illustrated in Plate II (Fig. 2A). The individual points on the chart are based on the average counts of 900 fruits each. The graph shows complete parallelism (except for treatment on February 23rd discussed above) of effect of treatment on advance of bloom and advance of maturation. The earlier the spray application, the greater the advance of bloom and maturation. Spraying on February 8th advanced maturity by seventeen days. While

there is a slight lag in advance of maturation behind advance of bloom with the earlier spray (no doubt due to lower temperatures earlier in the season), advances of maturation and blossoming by later sprays were identical.

Period of awakening.—Another aspect of the effect of spraying on bloom is that on the duration of the period of blossoming. Field observations seemed to indicate that while very early spraying tended to lengthen the period of bloom, late sprays considerably shortened it as compared with that of the unsprayed control. Actual records of beginning and end of bloom did not, however, support those observations. Differences of spread of bloom between sprayed and control plots as well as between treatments were small and not consistent. This

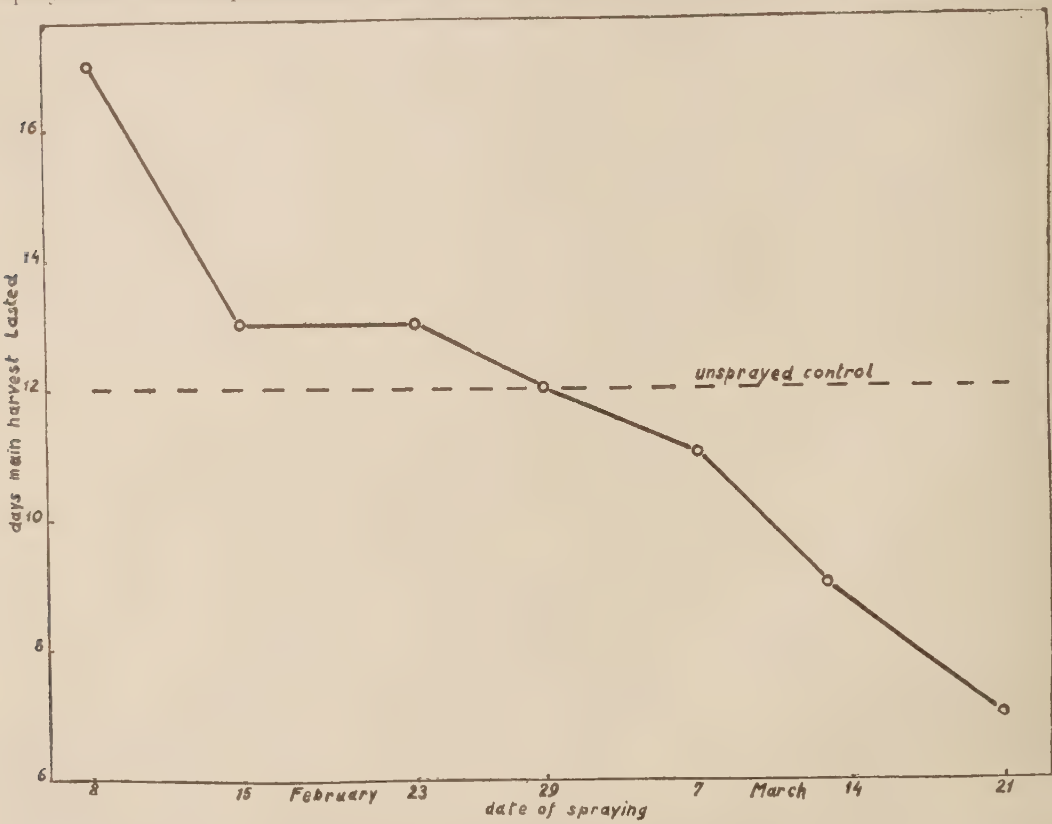

FIG. 3.
The effect of date of spraying on the spread of the main harvest of Red Astrachan apples. Mishmar Haemek, 1940.

divergence between visual impression and actual records might possibly be explained if it is assumed that some of the buds would have blossomed under all conditions and some of them opened only in response to treatment. If this hypothesis were correct and certain treatments were to effect a more contracted blossoming period, this would result in a curve similar in total range for all treatments (as recorded), but with a more pronounced peak for the effective treatments (as observed). This could be tested directly only by frequent and laborious blossom counts. In view of the fact, however, that (as shown above) dates of maturity and bloom are closely related, distribution of fruit maturing at different dates would be likely to indicate the situation at blossoming time.

The duration of total harvest showed a situation similar to the spread of bloom, i.e. inconsistent differences between treatments. But if the spread of the central half of the crop, i.e. between 25 and 75 per cent. of the number of fruits, was taken into consideration, a clear picture developed as shown in Fig. 3.

While it took seventeen days to harvest 50 per cent. of the crop on trees sprayed on February 8th, only a week was required with trees sprayed on March 21st, but twelve days with the unsprayed control. This curve tends to confirm the visual impression gained at blossoming time that early treatments tend to increase spread of main bloom, as deduced from the spread of the main harvest, while late application of the spray renders it more compact.

Amount of awakening.—Spraying not only stimulates earlier opening of those buds which, under conditions of prolonged rest, would be delayed in opening only temporarily, but also causes some of those buds to open which would otherwise remain dormant and unopened during the entire season, as shown in the first part of Table IV.

TABLE IV.

Effect of spraying on the opening of buds on one-year-old shoots, expressed as percentage of total buds. Mishmar Haemek, 1940.

Names of Varieties.	All opened buds.					Potential fruit buds only.				
			Difference.					Difference.		
	Control.	Sprayed.	Sprayed minus control.	Significant P=0·05.	% of control.	Control.	Sprayed.	Sprayed minus control.	Significant P=0·05.	% of control.
Banana ..	61·1	86·8	25·7	10·8	42·1	41·4	74·2	32·8	10·3	79·3
San Sauveur	50·6	84·7	34·1	13·3	67·4	37·7	72·0	34·3	12·5	91·0
Hammerstein	36·6	60·8	24·2	10·2	66·1	25·8	48·8	23·0	9·2	89·2
J. Lebel ..	31·7	49·1	17·4	—*	54·8	18·7	38·3	19·6	—*	105·0
Peasgood ..	25·3	59·7	34·4	13·5	136·0	20·6	48·9	28·3	10·4	137·0
Queen P. ..	18·5	40·6	22·1	10·3	119·0	9·4	28·2	18·8	9·3	200·0
R. Astrachan	17·3	46·6	29·3	12·6	171·0	8·8	33·9	25·1	10·8	285·0
Rome ..	17·8	54·6	36·8	9·0	207·0	5·9	41·6	35·7	8·2	604·0
Gravenstein	16·2	44·2	28·0	—*	173·0	6·5	30·4	23·9	—*	369·0
Delicious ..	13·8	43·6	29·8	15·1	216·0	5·7	30·5	24·8	10·4	435·0
W. Calville ..	15·0	58·7	43·7	16·2	291·0	5·1	45·2	40·1	14·1	788·0

* Only two trees were available for each plot.

It will be of interest to note the relatively small percentage of buds which opened on one-year-old unpruned shoots under conditions of prolonged rest (unsprayed control), in a number of commercial varieties. With half the number of varieties more than 80 per cent. of their buds remained dormant, while even with the variety Winter Banana, which produced the best leaf cover, only 61 per cent. of the buds grew.

The results reported for the sprayed plots represent the average of the three late treatments, applied March 7th, 14th and 21st, 1940, respectively. The effect of the spray on all varieties is very striking. While with four out of eleven varieties there was an increase of open buds by one-half above the control, in three other varieties over twice the number of buds opened ; and four varieties even more than trebled their open buds. There exists a definite trend indicating that the effect of the treatment on different varieties is inversely related to the percentage of buds which would open without treatment. Thus the differences between varieties with respect to the total number of open buds are, on the whole, smaller with sprayed trees than with untreated controls.

The date of spraying greatly influences the effectiveness of the treatment with respect to the number of buds which open, as shown in Figs. 4 and 5. Early spraying, while affecting the time of bloom does not increase the number of buds which open, but the effectiveness of spraying on the number of buds to open increases as the time of spraying approaches the date of normal blossoming. This was shown to be true for a number of varieties (Fig. 5) in 1940. But since these results showed a certain amount of fluctuation, tests concerning the effects of varying time of application on the opening of buds were carried out with from two to six varieties during four seasons in four localities, representing different climatic regions. Typical results obtained with Queen Pippin are shown in Fig. 4.

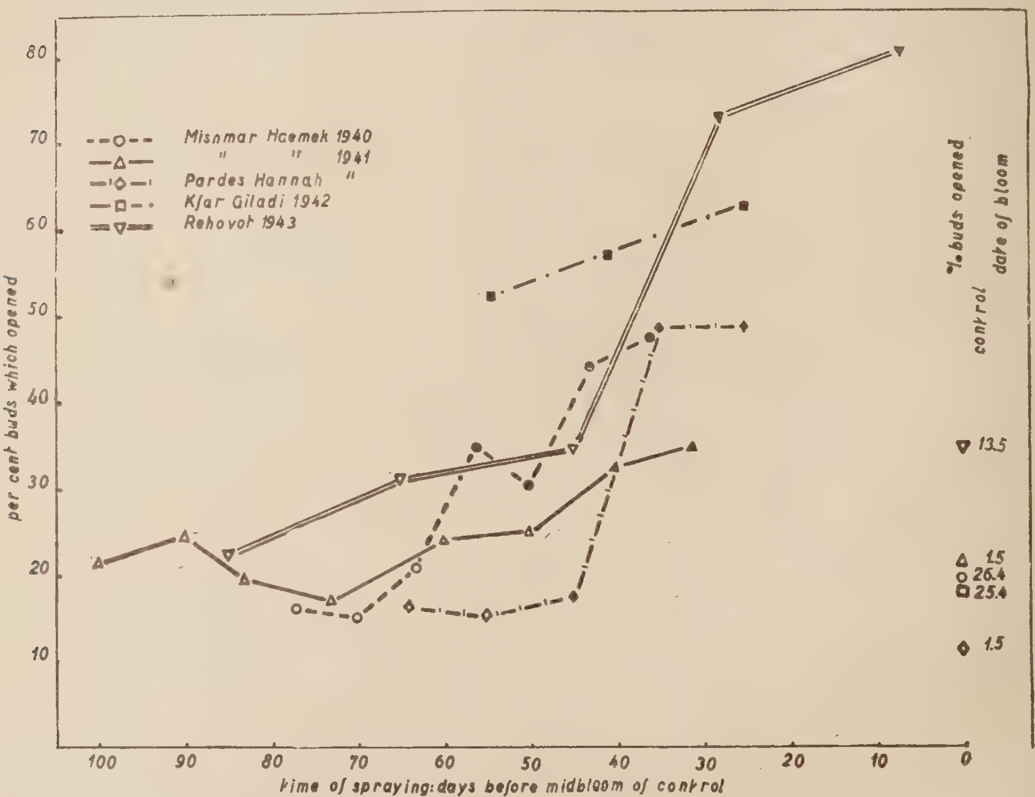

FIG. 4.

Effect of date of spraying on the opening of buds on one-year-old shoots of Queen Pippin.

Near the right-hand margin of the figure the reader will find the dates of mid-bloom of the controls next to the points which indicate the percentage of their buds opened. It will be noticed that the five curves obtained under different climatic conditions show a parallel trend, which strongly suggests that the response of buds to spray stimulus with date of spraying is of the sigmoid type. Those points on the curves which represent statistically significant (P=0·05) differences above the control are inked in full. They show that bud opening due to spraying is increased significantly if the treatment is applied at any time from seven to eight weeks before mid-bloom and until close to it. Results approaching the optimum were obtained

if spraying was carried out within a month before mid-bloom. A certain amount of spray damage (reduced size of leaves) was recorded only once with Delicious at Rehovot in 1943, when the last spray was applied after blossoming had actually started, while in a few other cases occasional scorching of leaf tips of advanced terminal growth was observed, the damage being of no consequence.

The development of the additional buds which opened as a result of spray stimulus was followed up during the summer immediately following the treatment. In September, the buds were grouped according to their development into four classes: (1) weak buds giving rise to one or two leaves; (2) potential fruit buds, under which term were included clearly developed fruit buds and buds having a well-developed rosette, but of which it could not be said with certainty, in all varieties, whether they would form blossoms; (3) spurs; and (4) shoots. The results of these counts are shown in Fig. 5.

Shoot and spur development was either unaffected by the treatment, or the effect (such as increased shoot growth due to late spray of Banana) was not consistent in different varieties. On the other hand, the effect of spraying on increase of leaf and fruit buds is evident in all varieties. It is clearly seen that most additional buds which open owing to spray stimulus develop into potential fruit buds. Therefore the percentage increase of potential fruit buds, as shown in the second part of Table IV, is higher than if it were only proportionate to the increase in total number of growing buds, as given in the first part of the Table. While this is true for all varieties, it is particularly striking for those varieties the buds of which would mostly remain dormant unless treated. Here the range is from a threefold increase of fruit buds over control, with only a twofold increase of total open buds in Queen Pippin, to a ninefold increase of fruit buds against a total fourfold number of buds opened in White Winter Calville.

While this increase in fruit buds would be expected to influence yields in the following season there is also an immediate effect on the number of blossoms and amount of fruiting during the season of treatment. This effect may be appraised with the help of Table V.

TABLE V.

Effect of time of DNC-mineral oil application on current season's fruiting. (Percentage of buds which matured fruit.) Rehovot, 1943.

Variety.	Control.	Sprayed on: Feb. 17.	Mar. 9.	Mar. 29.	April 16.	May 6.	Significant Difference (P=0·05).
Queen Pippin ..	1·2	3·4	2·2	5·3	14·4	13·0	5·6
Delicious	1·1	2·9	4·0	4·2	6·2	3·2	3·6

Each figure is based on four replicates of approximately one hundred buds each. Fruits and fruit clusters were counted after the June drop. The data are reported as percentage of buds which mature fruit, since the average number of fruits per cluster was similar for all treatments. In spite of the considerable variability which would be expected with this type of material, the increased yields obtained by spraying in April are highly significant, and the general trend of results obtained is similar to the trend in increase in opening of buds shown in Fig. 4 with the same trees. The reduction in the yield of Delicious after spraying on May 6th is due to spray damage; in this case bloom started on May 3rd.

While a fivefold or even tenfold increase in yield was thus obtained by late spraying of unpruned vegetative shoots, such large differences would not be expected with entire trees under commercial conditions, where a large part of the fruit is borne on spurs which seem to terminate their rest more readily than shoot buds. Moreover, in bearing trees, most of the

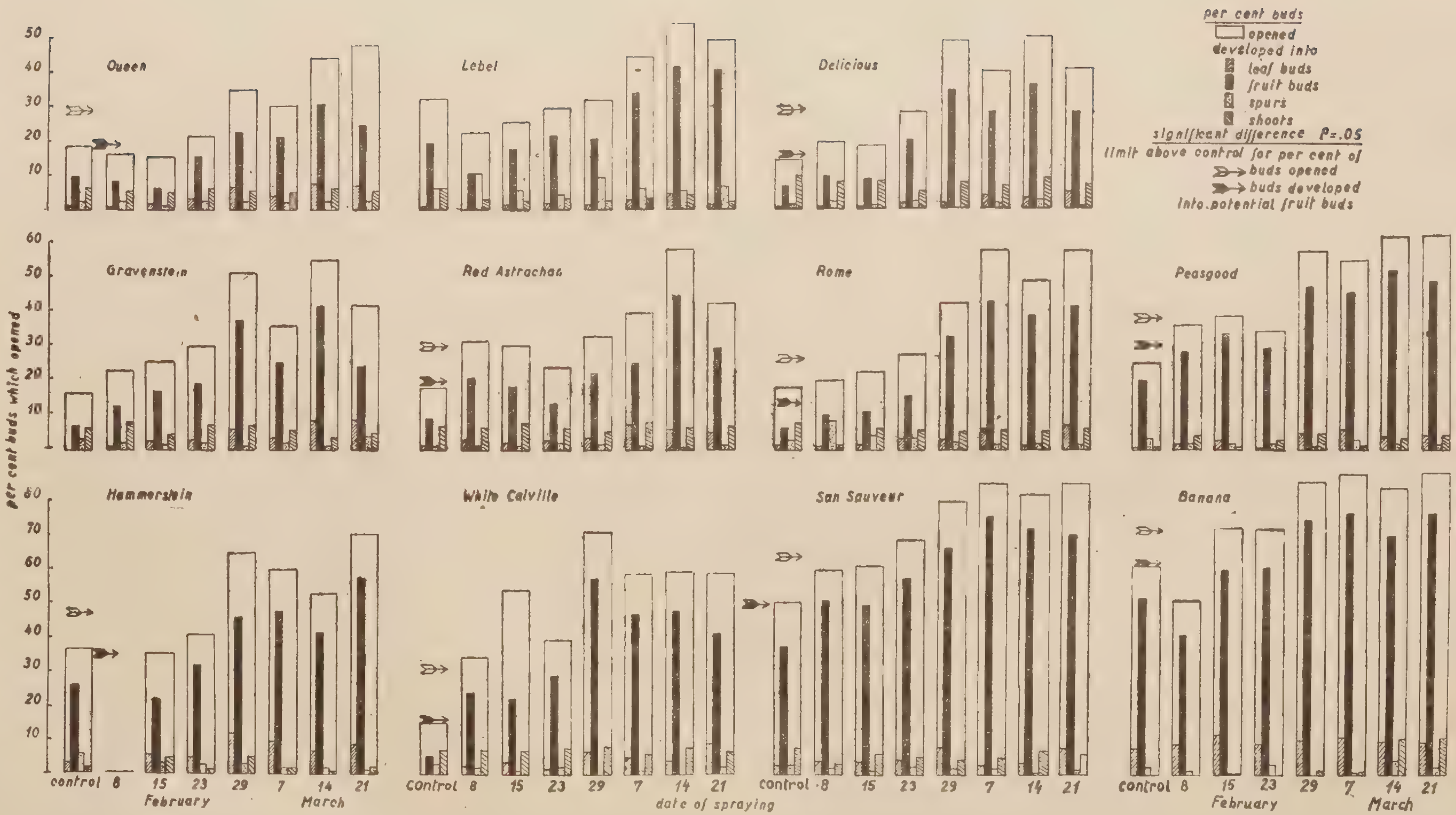

FIG. 5.

Effect of date of spraying on the number of buds which opened on one-year-old shoots and on their development during the season subsequent to treatment (expressed in percentage of total buds). Mishmar Haemek, 1940.

longer shoots are pruned, thus not only reducing their relative fruiting area, but also subjecting their distal part to the stimulation of the pruning wound. Furthermore, in commercial practice thinning of fruit would be expected considerably to reduce the effect of spraying on yield of varieties, particularly in years with heavy fruit set.

A long term commercial spray experiment was started in 1940 in an orchard planted in 1936. Unfortunately the experiment had to be discontinued after two seasons, for which the yield data are shown in Table VI.

TABLE VI.

The effect of DNC-mineral oil spray on yield of a young apple orchard. Tel Yosef, Esdraelon Valley.

Name of		No. of trees per plot.	Year.	No. of fruits per tree.		Difference (sprayed-control).	
Variety.	Rootstock.			Control.	Sprayed.	% of control.	coeffic. of variability.
San Sauveur ..	Khashabi	17	1940	173	172	−0·4	13·8
			1941	247	253	2·4	4·1
Jakob Lebel ..	,,	11	1940	24	38	58·3	30·0
			1941	46	102	120·0	18·3
Queen Pippin	Doucin	27	1940	70	105	50·0	17·0
			1941	146	310	112·0	14·1
Rome ..	,,	17	1940	91	228	151·0	12·4
			1941	144	246	70·8	17·2
Delicious ..	,,	10	1940	14	193	1280·0	21·2
			1941	246	238	−3·2	5·0

The yield response to spraying differed for different varieties, but all except Calville de San Sauveur showed considerable increases, the yield being from one-and-a-half times to twice the crop of the control trees. The varieties in this Table are arranged in the same order as in Table IV, where the names are listed approximately in the order of increasing effectiveness of the treatment in regard to the number of buds opened and to the formation of fruit buds at Mishmar Haemek in 1940. The same trend will be seen with yield at Tel Yosef. San Sauveur which even under conditions of prolonged rest opens a large percentage of its buds and usually sets heavy crops does not show any increase of yield due to spraying. Unfortunately, no data are available concerning the amount of fruit removed during thinning, so that it may very well be that, while spraying did to a certain extent increase the amount of blossoming and fruiting, this difference was obliterated during thinning.

With Jakob Lebel and Queen Pippin, which come next in their response to spray treatment, first year's crop increase resulting from the increase in opening of buds is not statistically significant. In the second season a doubling of the yield is found, resulting from the cumulative effect of increased fruit bud formation in consequence of the 1940 spraying, as well as the increased opening of buds due to the spraying in 1941. With Rome Beauty yield was more than doubled even in the first year. This variety usually has to be thinned very heavily, so that the increase by two thirds over the control in the second season may represent only a relatively small part of the fruit set. Delicious, the variety which had been shown to open the lowest percentage of buds on one-year-old shoots, showed the greatest response to spraying. Delicious comes into bearing usually in its sixth year, and its first commercial crop was advanced by one year as a result of spraying. Whether the lack of difference between sprayed and untreated Delicious trees in the second season was due to the large crop on the sprayed trees in the previous season, or to heavier thinning of a rather large crop considering the age of the trees, cannot now be ascertained.

DISCUSSION.

After having described separately the various aspects of response of apple trees to DNC-mineral oil spray, it is desirable to view the phenomenon as a whole. For this purpose the writer would like to make use of the terminology introduced by Mally (14), who, working on codling moth control, distinguished between a "forcing" action of mineral oils, which makes the trees foliate and blossom earlier than normal without eliminating irregularity, as opposed to the "normalizing" action of linseed oil, which causes regularity of bloom and foliation without forcing it into activity earlier than normal. The data reported above show that either result may be obtained by means of the same agent merely by changing time of application.

Although it may not be customary to introduce additional evidence into a concluding discussion, it would seem useful to allude to Fig. 6, Plate III, at this point, which illustrates fully the effect of the time factor. The control shows only terminal and basal growth, while most of the branch is naked. Notwithstanding the advanced date at which the photograph was taken, two blossom clusters may still be noticed in addition to the cluster of small fruits near the tip. The two branches which had been sprayed early in the season show only a slightly improved cover as compared with the control, but the large sized fruit, due to earlier bloom, testifies to the "forcing" effect of the treatment. The fruit on the branches which had been sprayed at later dates is only slightly more advanced than the fruit of the control, but most of the buds opened and developed to a uniform stage, which gives a "normalizing" effect.

In view of this it is difficult to find "a most effective" time of spraying, since time can be adapted to the effect desired. In certain cases "forcing" may be desirable. With early varieties an advance by over two weeks, as obtained in the experiments with Red Astrachan, will frequently mean a considerable advantage in marketing, far outweighing the somewhat increased picking costs due to extended harvest season. Forcing may also be resorted to in order to solve pollination problems by spraying the normally later blossoming variety. Furthermore, risk of damage to bloom by hot dry winds occurring in many subtropical regions in spring, may be reduced by spreading the blossoming over a longer period. This may be arrived at either by spraying early, or, better still, by forcing the bloom of only part of the orchard.

Late spraying, on the other hand, has certain advantages, although it was shown to have only a very slight forcing action. While early spraying tends to extend the blossoming and ripening period, late application tends to concentrate it. The more uniform ripening resulting from it represents a considerable saving with some varieties, such as Gravenstein and Astrachan, which otherwise have to be gone over for picking rather frequently. Concentrated bloom also facilitates codling moth control. The most important effect of late DNC-mineral oil spray, however, is a reduction of the number of buds which remain in a resting condition. This not only improves the status of varieties which, even without treatment, open a relatively large proportion of their buds after warm winters, but may permit the planting, under such conditions, of certain varieties which, because of their incomplete foliation, have been debarred from it in the past. The importance of increased opening of buds for the health of the tree in reducing sunburn and increasing the photosynthetic area is obvious. Since leaf rosettes became more numerous, the carbohydrate supply must certainly have been increased by the larger leaf area, and possibly by more efficient synthesis by spur leaves; and this increased supply was not offset by greatly accelerated growth, in view of the fact that shoot formation was not much altered. While this increase of the carbohydrate/nitrogen ratio (3) may not be desirable in all cases, such as in senescent trees or in the off year with alternate bearing varieties, it represents a great boon particularly for young orchards and shy bearers. The yield response, obtained even under commercial conditions of pruning and thinning, shows the cumulative effect of the stimulation of blossom buds to open and the increased fruit bud formation. This corroborates findings with oil sprays, on pear trees in South Africa (1), to the effect that one season's spraying is less effective than continued application for two seasons.

Because of its remarkable effect on yield, late spraying of apples with 3,5-dinitro-*o*-cresol-mineral oil emulsions must be classed among the major horticultural practices in areas affected by prolonged rest. It has the additional advantage of being a double purpose spray, since the same material is used in insect control—an important factor in reducing cost of operation. This spray has been used commercially throughout Palestine for the past four seasons, and the importance (for the opening of buds) of even relatively small differences in date of spraying became, in certain cases, even more apparent in the field than was shown in the experimental plots. In view of the very large annual fluctuations in date of blossoming, which it was found almost impossible to forecast (because it depends on at least two conflicting factors—cold winters which hasten bloom, and cool springs which delay it) it became important to connect the date of spraying with the development of the tree. It was found that the spray could still be applied at the stage when the most advanced fruit buds were at " bud burst " (with bunch of curled leaf-tips protruding), a practice which had also been found by Steer and Moore (19) to result in no commercial damage. In cases where some of the terminal buds or those which had been stimulated by early pruning wounds had already progressed further, the marginal- or tip-burn of those leaves was of no importance when compared with the accrued advantages.

If the effects of delayed spraying are summarized it is found that they correspond to what Mally calls " normalization ". The abnormal behaviour of apple trees following warm winters consists of : delayed bloom and foliation, irregular and protracted opening of buds, some of the buds remaining in the resting stage, and of bloom preceding foliation (4). While the delay in opening of buds could not be corrected by late spray application, because of the closeness of date of treatment to date of natural bloom and foliation, all other phenomena were nearly brought back to normal. It might also be questioned whether, if all other abnormal growth phenomena were corrected, merely a later date of opening of buds could be called abnormal. It would become normal for the particular climatic conditions. The modifying effect of time of application on amount of " normalization " might possibly be explained by unequal susceptibility to the stimulus on the part of different buds. While only few buds had progressed far enough towards the completion of their resting stage to be susceptible (22) to the early spray stimulus, more buds might have been receptive at a later date. If this is true, then both forcing and normalizing are different aspects of one and the same mechanism. It might then be expected that if a sufficiently strong stimulant could be found, complete normalization might ultimately be approached by controlling delayed opening of buds, together with other irregularities, by spraying earlier in the season.

Still, if the delay in opening of the buds and the other phenomena are conceived as symptoms of one abnormal state, prolonged rest, the gravity of all those symptoms would be expected to increase similarly and in proportion to the chilling requirements of a certain variety. Thus, the varieties Winter Banana and Calville de San Sauveur have a low chilling requirement and succeed even in regions with such warm winters that no other European variety has so far been found to grow in them successfully. As shown above they are the first to blossom, can be most easily stimulated by early spray application and open the largest percentage of buds among all varieties studied. As against this, the data presented for a number of other apple varieties do not always show the expected parallel trend. Neither does the variety's character of earliness of blossoming always go parallel with high percentage of buds which open, nor the spray response which is coupled with them. Thus, Delicious and Red Astrachan which in most seasons belong to the early blossoming varieties show a very good spray response and could thus be thought to have a light rest, if it were not for the fact that most of their buds remain dormant, which is a definite sign for trees with a high chilling requirement. Such cases would seem to indicate that the question whether prolonged rest is one single phenomenon or involves more than one mechanism, cannot as yet be answered with certainty, and that the chilling requirement of varieties may be a complex rather than a simple property.

In conclusion the writer wishes to express his gratitude to Dr. Y. Carmon for his

collaboration in this work, to the Shell Company for providing the spray material and co-operating in many other ways, and last, but not least, to the various growers who spent a great deal of effort in co-operating in the experiments.

SUMMARY.

1. Apple trees growing in Palestine, with winters not cold enough to break their rest normally, were sprayed with 3-5-dinitro-*o*-cresol-mineral oil emulsion. Its effect on time of opening of buds and on maturation of the fruit was recorded. The number of buds which opened on one-year-old shoots was counted and their further development followed up.

2. The spray material was shown to be effective in breaking prolonged rest of apple trees. The effectiveness of the material changed both qualitatively and quantitatively with date of spraying.

3. Early spray application had a "forcing" effect, stimulating earlier foliation, bloom and maturation. This effect diminished with delay in date of spraying.

Late spray applications had a "normalizing" effect which decreased with earlier date of application. The number of buds remaining in the resting stage was reduced. A number of the additional buds which opened were blossom buds, which increased the yield in the same season. Most of the additional vegetative buds developed into fruit buds, so that the yield in the second season of spraying showed a residual effect from the previous one, in addition to the current season's increase. The period of main bloom and ripening was shortened and time of foliation moved close to time of bloom (bloom considerably precedes foliation under conditions of prolonged rest).

4. "Forcing" was more successful with varieties which naturally bloom earlier. Thus, total spread of bloom between varieties was increased, though the sequence of blossoming was not altered.

A higher relative amount of "normalization" was obtained with varieties showing a larger number of resting buds. Thus this treatment, by a relatively larger increase in the opening of buds of those varieties with the larger number of dormant buds, reduced differences between varieties.

5. Practical and theoretical aspects of the results are discussed.

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PLATE I.

FIG. 1.

The effect of spraying on development. Delicious trees at Givath Brenner (Southern Coastal Plain) on April 30th, 1931. Untreated control, dormant ; trees sprayed on March 12th, fully foliated.

ERRATUM

Journal of Pomology & Horticultural Science, Vol. XXI, Nos. 1-4. The Use of Dinitrocresol-Mineral Oil Sprays in Apple Orchards, by Rudolf M. Samisch Plate I (*facing page* 178)

Date should read 1939 *not* 1931

PLATE II.

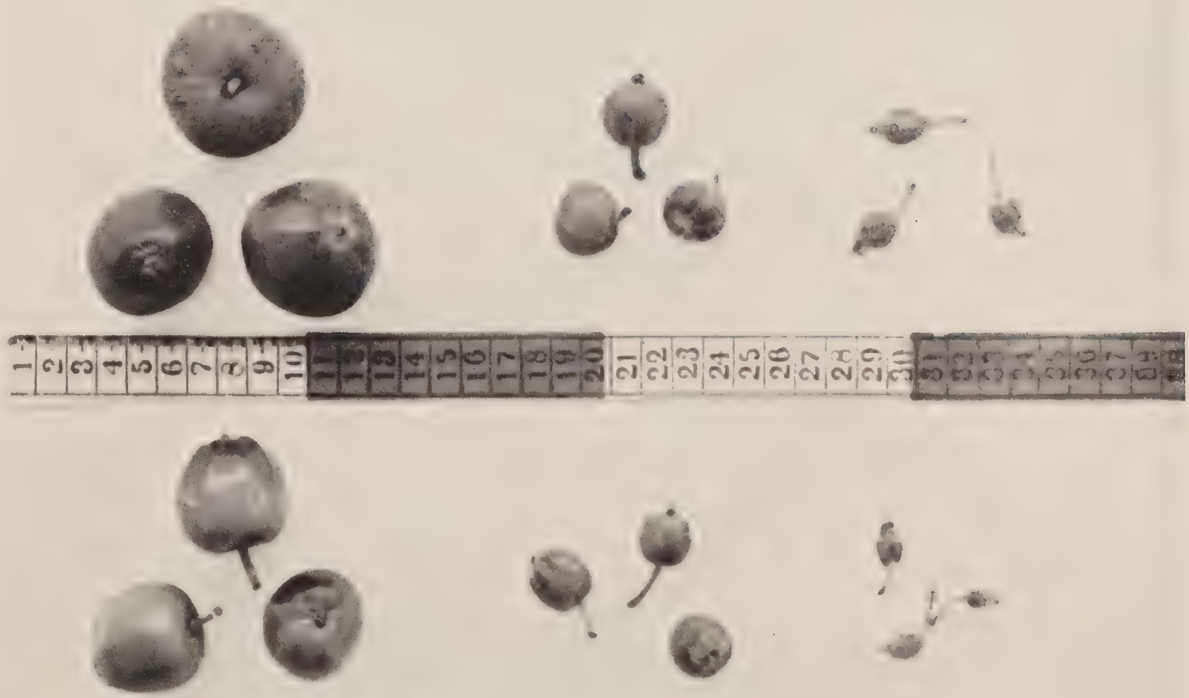

FIG. 2A.

Effect of time of spraying on maturation (photographed at Mishmar Haemek on May 12th, 1941). Upper row: Red Astrachan; lower row: Calville de San Sauveur; from left to right: sprayed January 31st, March 6th; unsprayed control.

Fig. 6.

Effect of DNC-mineral oil application on one-year-old shoots of Queen Pippin. Rehovot, June 6th, 1943. From left to right: untreated control; treated: February 17th, March 9th, April 16th, and May 6th.

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PERENNIAL CANKER OF APPLE TREES IN ENGLAND

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Introductory and historical.

In the autumn of 1941 apple branches affected with severe dieback and canker were received by the writer for investigation. A species of Gloeosporium was readily isolated from the two types of lesions (13) but could not be ascribed to any species already recorded in the British Isles. The same fungus had previously been isolated from a canker of Early Victoria apple and from dieback lesions following summer pruning cuts of apple trees at Cropthorne, Worcestershire, and at Long Ashton. From 1937 to 1940 the same fungus was frequently found causing a circular lenticel rot of apple fruits in cold storage at Long Ashton. It has now been identified as *Gloeosporium perennans* Zeller and Childs (15), a species not hitherto recorded in this country, which is responsible for the disease known as Perennial Canker, and for a storage rot called Bull's Eye Rot (6), in the U.S.A. and British Columbia.

G. perennans was first described in the U.S.A. in 1925 (15) when the disease it causes was distinguished from apple tree Anthracnose, caused by *Neofabraea malicorticis*. Infection occurs through pruning cuts, Woolly Aphis punctures and other bark injuries, and cankers are produced which may increase in size for several years with the production of six to seven concentric rings of callus. The fruit rot was found not to be serious, being usually well controlled by the adequate orchard spraying given. More recently, Brien (2) has stated that the so-called Delicious Spot of apples, in New Zealand, is caused by *G. perennans*. In 1929, Childs (4) described this fungus as a wound parasite producing lesions in close association with late Woolly Aphis attack, the cankers advancing most rapidly after exposure to winter temperatures of -2° to -27° F. He found that the cankers were not invariably perennial and that new annual infections develop only when conditions are favourable.

Cooley and Shear (5) found that the disease had a very limited range in North America, and they established a definite correlation between the occurrence of the Canker and winter precipitation only in areas where the temperature was sufficiently low. They found the fruit rot to be severe in one locality only, where late rains and spore discharge occurred before harvest. Brown (3) concluded also that Perennial Canker was primarily due to winter injury, with Woolly Aphis attack as an accessory factor. Reeves *et al.* (11) observed extensions of infection from cankers treated experimentally with wound dressings of various vegetable oils containing copper arsenite.

In 1932, Miller (10) made physiological studies of *G. perennans* and *N. malicorticis* and found that the two fungi reacted similarly to both temperature and acidity of the nutrient medium, but *N. malicorticis* showed greater retardation of growth in tannic acid media than *G. perennans*. Finally, Kienholz (7) states that although the two fungi cannot be distinguished by morphological, physiological and mycological characters yet the two natural cankers caused by them are quite distinct. He suggests that the two fungi may have arisen from a single species by mutation, and he retains *G. perennans* as a distinct species but transfers it from the genus *Gloeosporium* to *Neofabraea*.

Name of the disease.

Because of the supposedly persistent nature of the branch lesions Zeller and Childs named the disease Perennial Canker (15), and although subsequent workers have shown this to be to a large extent a misnomer yet the name has been retained and will be used here to prevent confusion with our better-known apple Canker caused by *Nectria galligena* Bres. (8).

As already stated the fruit rot caused by *G. perennans* is known as Bull's Eye Rot in the U.S.A., and Delicious Spot in New Zealand; but in this country it is impossible to distinguish it readily from Bitter Rot caused by *G. album* Osterw. and by *G. fructigenum* Berk. The writer, therefore, suggests that the disease of the fruit should also be included under the name Bitter Rot, thus adding a third species of Gloeosporium to the two already known to cause this disease.

G. album and *G. perennans* are very similar in many respects and so are the types of rots they produce in the fruit. Their methods of spore formation, rates of rotting and growth in culture are practically alike, and they can be distinguished only by a study of their spores and their cultural characteristics. The spores of *G. album* are hyaline, non-septate and distinctly curved measuring $12\text{-}27\mu \times 4\text{-}5\mu$ and are readily distinguished from those of *G. perennans* which are hyaline, non-septate and mainly ellipsoidal, measuring $4\cdot5\text{-}21\mu \times 1\cdot5\text{-}6\mu$. *G. fructigenum* differs from the other two species by its more rapid rate of rotting, the lesion being darker in colour and characterized by the production of greyish-black surface mycelium and glutinous masses of spores which are orange in colour. Inoculations made by the writer indicate that *G. album* and *G. fructigenum* are both non-parasitic to apple branches whereas *G. perennans* is an active wound parasite of them.

FEATURES OF THE DISEASE.

(i) *On branches.*—The most serious aspect of Perennial Canker in this country is the dieback resulting from the invasion of summer pruning cuts (Fig. 1, Plate I). The lesions extend from ½ to 6 in. behind the cuts, deeper penetrations being usually found on older branches. This dieback progresses rapidly along the branches causing longitudinal splitting and peeling of the bark thus exposing the swollen, brown cortical tissues which eventually dry out and become black. The limit of the lesion along the branch is marked by a groove-like ring of callus, laid down usually by the end of November but frequently delayed until early spring. This often seals off the diseased from the healthy tissues permanently; but quite frequently lesions have been observed with from two to four irregularly spaced callus rings, each indicating an unsuccessful attempt by the host to prevent further backward extension of the lesion.

Cankers are formed by penetration of the fungus into all types of bark injuries especially those due to Woolly Aphis, but they also arise by extension of dieback of a lateral branch into the stouter member which bears it. Bark peeling is not so pronounced in cankers and is most conspicuous around the swollen margin of the lesion. Cankers are elliptical, slightly sunken and surrounded by a ring of callus (Fig. 2, Plate I). No cankers were observed having many concentric rings of callus, as described by Zeller and Childs (15), except in a few isolated cases where attack by Woolly Aphis had resulted in the spread of infection beyond the original callus ring and such spread had become limited by a second callus ring.

Sporing bodies, or acervuli, arise below the bark as small, grey pads which finally burst through it or remain on the exposed cortical tissues where, under moist conditions, small white masses of spores are produced.

(ii) *On fruits.*—Bitter Rot caused by *G. perennans* is essentially a storage rot in England, but it has been identified once among lenticel rots of Allington Pippin before picking, in early October. In cold store it normally appears in November and December, when small circular, flattened or concave lesions appear, each having a lenticel at its centre. This centre is usually pale brown and is surrounded by a darker brown marginal zone, but lesions showing no colour zonation are frequently found on the coloured side of the fruits. Individual lesions rarely exceed 1¼ in. in diameter and the depth of penetration into the flesh is approximately equal to half the surface diameter (Fig. 3, Plate II). Acervuli, originating below the skin, burst irregularly through it and produce white masses of spores embedded in mucilage. Storage losses as the result of attack by *G. perennans* are slight. During the storage seasons 1937-38 and 1938-39 only 0·03 and 0·01 per cent. respectively of a large number of apples examined

were attacked by this fungus. In contrast, during the same two seasons, losses caused by *G. album* were about 15 and 8 per cent. respectively. Recently, however, apples infected with *G. perennans* have been found more frequently, and the fungus has been isolated from fruits grown in Somerset, Warwickshire, Worcestershire, Cheshire, Kent and Cambridgeshire, so that its distribution in England is evidently widespread.

The fungus.

An intensive mycological study of the fungus has been made, but it is not possible to describe the details of this work here, and a brief summary of it must suffice.

G. perennans has been isolated in pure culture from single spores derived from the fruiting bodies (acervuli) produced on cankers, dieback lesions and rotted fruits. The isolations from material obtained in different parts of the country have all yielded the same fungus, and comparison of the types of spores produced (macro- and microconidia) with those described in America for *G. perennans* showed close agreement, especially in the formation of secondary or microconidia. Controlled infection experiments have been carried out and the fungus recovered in pure culture from the lesions produced. All lesions produced by inoculation were of the "simple" type, i.e. the lesion was bounded by one marginal callus layer, but spread beyond this was obtained when the limiting callus layer was injured artificially. The fungus is essentially a wound parasite, but though it cannot penetrate the unbroken skin of the fruit it can gain entrance to it through a lenticel. The rate of growth of the fungus and the rate of decay it causes in the fruit have been carefully compared with those of *G. album* and *G. fructigenum*. In this respect *G. perennans* closely resembles *G. album*, the rates being considerably slower than that of *G. fructigenum*.

In addition to the production of acervuli, the fungus has been found to produce in culture, black bodies strongly suggestive of immature apothecia, which would constitute its perfect stage of fructification; but no unmistakable asci or ascospores have been observed. Such bodies were also reported by Kienholz (7). Moreover, he reported the discovery of the perfect stage occurring naturally on the cankers. These have, so far, not been observed in England.

Perennial canker in relation to pruning.

G. perennans has been isolated from branch lesions resulting from infection of summer pruning cuts from three separate localities. In one instance serious and widespread damage was being caused, and since no case of Perennial Canker had been reported hitherto in England detailed observations were made of this outbreak.

The orchard at Bidford-on-Avon, Warwickshire, of five to six acres, had been planted eight years previously on land already heavily manured for vegetable cultivation. Laxton's Superb and Cox's Orange Pippin trees were growing in rows 15 feet apart as dwarf pyramids. In 1940 the trees were summer pruned, and a slight amount of dieback was then observed. The pruning was repeated in 1941, and a few weeks later a very high proportion of the cuts had become infected, the lesions continuing to extend down the branches until December, when their further development was retarded. By April, 1942, the lesions had become callused off and their extension completely arrested. Later in the season, however, a considerable number of the dieback lesions showed extensions beyond the callus barrier; acervuli were sporing on the lesions and apparently serving as sources of further infection, for many cankers were developing on branches showing any form of mechanical or Woolly Aphis injury.

In one part of the orchard, however, trees of the same varieties growing under grass, planted at the same time, but not summer pruned, were completely free from attack. To ascertain whether grassing down or summer pruning influenced the occurrence of the disease, four trees in the part of the orchard under grass were pruned on July 1st, 1942, each cut being made immediately above a bud, and 100 cuts on each tree were marked for future observation. When

examined on September 23rd, 1942, a count of the 400 marked cuts showed that 73 per cent. had become infected with a dieback, the lesions varying in length from ½ to 1½ in. Each infected cut was readily detected by the presence of a dead leaf, situated immediately below the cut, which had been killed as the dieback progressed along the branch. A random sample of the lesions when incubated, produced spores from which *G. perennans* was isolated in a pure state.

Summer pruning carried out in late July, 1943, on Cox's Orange Pippin, Allington Pippin, King Edward and Early Victoria growing under grass at Long Ashton gave an infection count of 62 per cent. on October 13th, 1943.

A further experiment was made in the Warwickshire orchard to investigate whether "good" and "bad" pruning cuts had any influence on the incidence of the disease. 250 of each type were made on July 1st, 1942, and marked, the "good" cuts being made immediately above a bud, the "bad" cuts immediately below, leaving a long pruning snag. Infection counts made on September 17th, 1943, showed that 80-90 per cent. of both types had become infected and had died back between ¼ and 1½ in. A random sample of the lesions when incubated showed them to be infected with *G. perennans*.

In the course of winter pruning, carried out at Bidford-on-Avon and Long Ashton during the past two years, no infection has occurred through pruning cuts, the majority having become callused over by the following spring. Some of the cuts failed to heal normally and showed shrinkage and browning, extending backwards to ½ in., but *G. perennans* could not be isolated from any of them.

Control of the disease.

Spraying and wound dressings employed in the U.S.A. and British Columbia have given little success in controlling the disease (9). In England experiments have shown that *G. perennans* does not attack branches through winter pruning cuts, and since in all three of the cases studied the disease clearly originated from the infection of summer pruning cuts, a simple method of control, viz. the substitution of winter for summer pruning, suggested itself. This idea was tested in the dwarf pyramid orchard in Warwickshire, and as the successful cultivation of this type of tree is dependent on summer pruning it was first necessary to convert the trees to the bush type in which winter pruning is practised. The transformation was completed during the winter of 1941-42, and it included the removal and burning of all infected branches. The large number of winter cuts made were kept under observation, but by September, 1943, no signs of dieback or canker originating from these cuts were evident ; a very high proportion of them had become completely callused over.

DISCUSSION.

After a study of the factors which might be responsible for the occurrence and continued spread of Perennial Canker, McLarty (9) concluded that *G. perennans* existed in two forms. Firstly a parasitic one which causes fruit rot and branch cankers. The cankers develop in the autumn through bark injuries, their enlargement being arrested in the following spring by the formation of a callus. Thereafter the fungus exists in the cankered tissue in a second or saprophytic form ; sporing bodies are then produced and the fungus remains viable for periods up to three years, but it is incapable of renewing its parasitic role unless reinoculated into the living host tissue. Such reinoculation through the limiting callus layer is caused by feeding punctures of Woolly Aphis and by injury following low temperature, the greatest extension of individual cancers occurring after temperatures from $-2°$ to $-27°$ F. have been experienced (4). Hence in North America perennial cankers showing concentric rings of callus are formed, because *G. perennans* exists alternately in the parasitic and saprophytic states. Such cankers

might, therefore, be regarded as compound lesions, as opposed to simple cankers in which there is only one callus ring.

The numerous cankers examined in two English orchards have been mostly of the simple type with no extension of the fungus beyond the limiting callus layer. In a few isolated cases, however, where Woolly Aphis colonies were seen feeding on the callus tissue, extensions of the lesions occurred which, in turn, became limited by a second callus layer, and the cankers became of the compound type. Again, in controlled infection experiments (details of which will be given in a later communication) inoculation of injured apple branches, whether made in the spring or the autumn, produced simple cankers only, which still remained in this condition after two years. Furthermore, development of simple into compound canker lesions was obtained artificially in six out of eight cankers by scraping the callus layer with a sterile needle, thus enabling reinfection to take place. The formation of perennial cankers as a result of low temperature injury is most unlikely to occur under English conditions, but it cannot be totally disregarded as serious frost injury to trees does sometimes occur, as reported by Wallace (12) in 1929, when screen temperatures of 7° and 9° F, caused severe bark splitting at Long Ashton. Frost injury to the bark of apple trees in England was also recorded in 1917 (1).

Dieback resulting from infection through summer pruning cuts is the most serious aspect of the disease so far observed in England, and pruning experiments in infected orchards have shown a high incidence of infection through summer cuts even when the pruning was done satisfactorily. After developing during one season, the dieback lesions on the older branches become efficiently sealed off by a ring of callus, but on one- to three-year-old branches such rings in many cases appear to be inefficient, and extension of the dieback has been observed during the three or four subsequent years. The fungus can always be isolated from the proximal region of those lesions, which indicates that in these cases *G. perennans* remains viable in them for several years. In contrast, however, winter pruning cuts made in the same orchards showed no infection and callused over in a normal manner.

All the three outbreaks of Perennial Canker studied by the writer originated from dieback infection of summer pruning cuts. This fact and the evidence obtained from pruning experiments would suggest that similar outbreaks are most likely to occur in orchards where fruit is grown on an intensive system which usually involves summer pruning. Bitter Rot of apples caused by *G. perennans* has been found in six widely separated counties infecting apple fruits many of which matured in orchards where summer pruning was not employed. This suggests that the fungus has a widespread distribution in England and is present in many orchards, but as yet is causing no conspicuous branch lesions. So long as winter pruning alone is practised in such orchards the disease is not likely to make headway in them.

SUMMARY.

Perennial Canker caused by *Gloeosporium perennans* Zeller and Childs, and not previously recorded in England, has been found in three orchards causing canker and dieback of apple branches following summer pruning. The same fungus was isolated from lenticel rots of apples grown in six counties in the South and Midlands, showing that it is fairly widely distributed here.

A description of the disease on branches and fruits is given and it is suggested that the fruit rot be included under the name of Bitter Rot, one which already covers the fruit rots caused by *G. album* Osterw and *G. fructigenum* Berk.

The growth of natural and induced cankers continues, as a rule, for one season only and is then arrested by a callus layer ; extension of the fungus beyond this layer was observed only when it became injured mechanically or by the activities of Woolly Aphis. Numerous dieback lesions on one- to three-year-old branches showed yearly extensions behind the callus ring, and *G. perennans* was found to remain viable in the invaded tissues for several years.

PLATE I.

Fig. 1.
Dieback resulting from invasion of summer pruning cuts by *Gloeosporium perennans*.

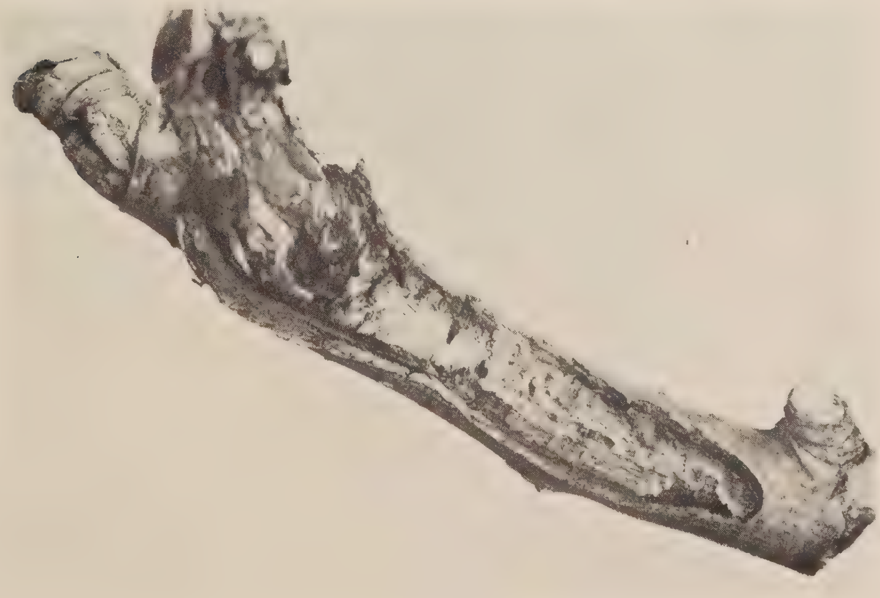

Fig. 2.
Canker caused by *Gloeosporium perennans*.

[face p. 184

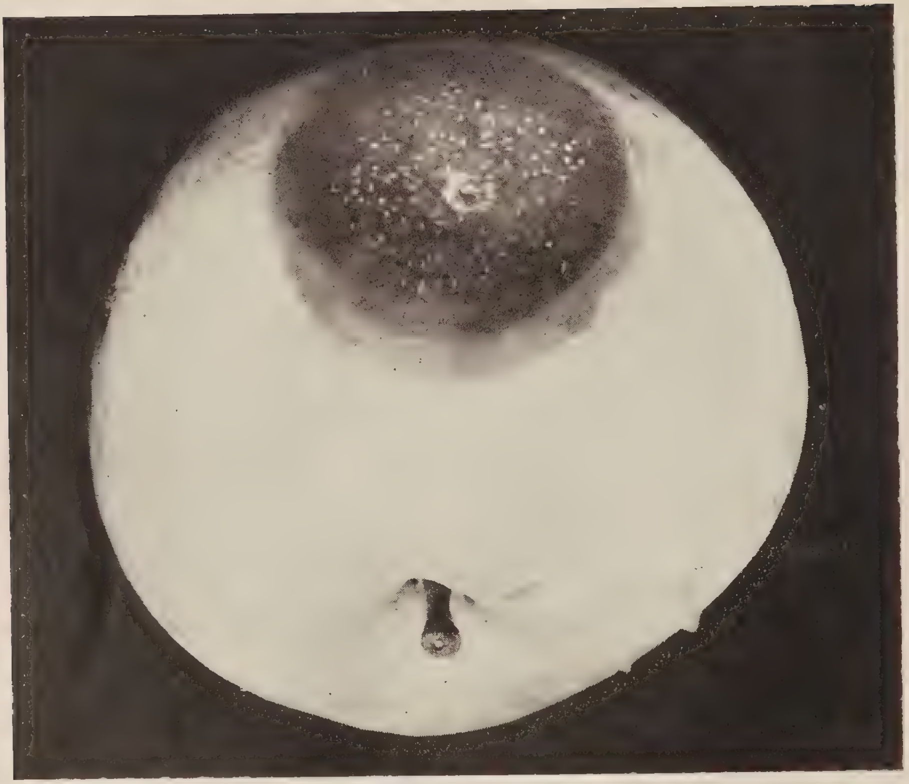

FIG. 3.
Bitter Rot lesion in Allington Pippin apple caused by *Gloeosporium perennans*.

Inoculation experiments have shown that the fungus is capable of penetrating bark only through injuries, whereas it can cause rot in apples through lenticels as well as through skin wounds.

Pruning experiments have shown that infection takes place through summer but not through winter cuts. One serious outbreak of Perennial Canker in England has been surmounted by a change over to winter from summer pruning. Even in orchards in which the fungus occurs on the fruits, it is considered that the disease is not likely to do serious damage to the trees, provided summer pruning is avoided.

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BOOK REVIEWS

OCCASIONAL PUBLICATIONS ON SCIENTIFIC HORTICULTURE, Nos. 1-3 (1939-42). Abridged edition with Supplement. December, 1943. (Published by the Horticultural Education Association. Publ. Editor, C. E. Hudson, Midland Agricultural College, Sutton Bonington, Loughborough, Leics. 4s. 3d. post free.)

On the outbreak of war the Horticultural Education Association reluctantly decided to suspend publication of its journal, *Scientific Horticulture*, in view of the obvious difficulties in the gathering of material and in printing. This decision was disappointing to a growing circle of readers who had come to look upon the journal as an increasingly useful and informative publication. The revival, in 1941, of the policy of holding annual conferences at which papers were read that were of interest and importance to a wider circle than those attending the meeting, induced the Association to print these and some other articles of informed comment as Occasional Publications for circulation to members only.

The first three of these are now made available to the public in the form of a collected volume. Publication No. 3 contains the text of the Association's Memorandum to the Luxmoore Committee on post-war agricultural education, and the opportunity was taken in this reprinting to issue also the Association's review of the Luxmoore Report in relation to horticulture.

Publication No. 1 is noteworthy for a championing of the Ministry of Agriculture's Grow-more Bulletin No. 1, which met with some adverse criticism on its first appearance. No. 2 contains a valuable article on home saving and storage of seed, by D. Boyes, while No. 3 consists of a series of papers on wartime vegetable and fruit growing read at the Association's Conference in the autumn of 1942, and also a most useful reference list and review of films of horticultural interest available up to November, 1943.

To those concerned in the problem of post-war agricultural education the most interesting parts of this volume will be the two memoranda previously mentioned. Together they constitute a statement of the policy of the Horticultural Education Association, a body representative of the whole field of advisory and teaching work in horticulture. It is not possible to consider even the main outlines of this policy in the short space of a review, but it may be said that, if adopted, the proposals of the Association would constitute a charter for horticultural education which would raise the profession to its proper status, an equal and co-operating partner with its brother profession of agricultural education.

R.H.S.

THE PRODUCTION OF SEED OF ROOT CROPS AND VEGETABLES. (Imperial Agricultural Bureaux Joint Publication No. 5, July, 1943, pp. 95, 3 pl., 2 figs., 3s.)

In this collection of articles, most of the available information on the subject has been brought together. The contributions are mainly written by authorities from the leading Research Stations and Government Departments in the various countries about which information is provided.

As a result of the war, seed production has had to be undertaken in many new areas with the consequent adaptation of methods of production, varieties, etc. This publication describes these new developments in as many countries as is possible from the information available under the present abnormal conditions, including England, Scotland, the Netherlands, Sweden, the United States of America, Canada, Australia, New Zealand, South Africa and the Colonial Empire.

In a number of cases the production of seed of root crops and vegetables has been attempted only during the last few years, and the information available is necessarily very limited : thus,

as suggested in the text, the articles are only progress reports. In other cases, such as seed production in England, the production of sugar beet and other seeds in the United States and Canada, detailed information is given.

From the reports given it is clear that there is a possibility of producing European vegetable seeds in many of the Dependencies. This is frequently most easily undertaken on the highlands in tropical regions, but whether this project will be worth developing will probably depend on the availability of a market after the war.

Those interested in seed growing in the established areas will find a concise account of the methods of production, the main varieties grown, and the organization and legislation related to seed growing. No attempt has been made to give comprehensive lists of references to literature on the subject, but references are given to a few of the main available sources of information. There is an excellent index. Readers requiring further information on special points are invited to apply to the Imperial Agricultural Bureaux.

It is hoped that this publication will be followed by others of a similar character on the same subject, as and when new information becomes available ; in the meantime, it forms a most useful source of reference to the latest information on the production of seed of root crops and vegetables.

C.E.H.

POTATO COLLECTING EXPEDITIONS IN MEXICO AND SOUTH AMERICA. II. Systematic Classification of the Collections. By J. G. HAWKES. (Cambridge, Imperial Bureau of Plant Breeding and Genetics, 1944, pp. 142, 7s. 6d.)

No food plant has received more attention during the last forty years from both scientists and breeders than has the potato. In the main, attention has been concentrated on the improvement of the domestic stocks of the plant : its capacity for producing varieties improved both in quality and yield, its immunity from Wart disease and its resistance to Blight and virus diseases. Although about a score of wild species were already known, it was not till work at Barley on *S. edinense* and *S. demissum* before the last war (which disclosed the inheritance of resistance to Blight) that any sustained effort to improve our cultivated potato by their use, was attempted. Indeed very few people in the United Kingdom, or outside it, were sufficiently conversant with any of the wild tuber-bearing species of Solanum to distinguish one from another ; and still fewer knew that some possessed qualities the inclusion of which would be of value in the make-up of a cultivated variety.

The Russian expeditions to Mexico and South America between 1925 and 1932 opened up vast new vistas. Our knowledge of the whole family was, at a stroke, enlarged, and the possibility of using the new material to breed varieties endowed with qualities as yet undreamt of, swam into view.

The work was brought a definite step further when, in 1938-39, Dr. J. G. Hawkes in company with Dr. W. Balfour Gourlay and Mr. Edward K. Balls set out on an exhaustive search for tuber-bearing Solanums throughout Mexico and the whole Andean Range of South America. More than a thousand specimens in the form of flower, seed, living tubers, and herbarium preparations were collected. The value of their work will be appreciated by the fact that Dr. Hawkes' taxonomic studies have revealed the existence of five cultivated species and 31 wild ones hitherto unrecorded, and has thus brought the known forms of tuber-bearing Solanums up to 20 cultivated and 150 wild species.

Hitherto we have had no complete description, and still less an analysis, of the qualities of the *Hyperbasarthrum* section of the genus. Hawkes has now provided us with the description of its members, together with invaluable notes as to their habitat and relationships—a service in this particular sphere, the like of which has not been rendered since Bitter's work of 1911-13, with which it agrees in quality, whilst greatly surpassing it in extent.

Ever since the Russian expeditions, plant breeders have been craving for some logical and reliable guide by which to find their way in a field which threatened to grow in confusion as it increased in extent. In Hawkes' work they will find much of what they need, the harbinger of still more to come. Whether his new Section *Cuneoalata*, and all of the 31 new species distributed throughout the other sections, will stand the test of time, has yet to be proved. It has not seldom proved easier to formulate a specific form than later to defend its existence. Be that as it may, future workers will have no difficulty in assuring themselves of the characters by which he has identified them, and their relation to types already known. Within a framework based on observation in field and laboratory, we now have a great mass of co-ordinated material which is more than a *catalogue raisonné* of the potato family. Wherever possible, the chromosome counts and such outstanding physiological characters as are known to be possessed by the different species, are given, not only of those occurring in his new material but also of those included in former collections.

Useful Tables will be found, showing the incidence of the different somatic outfits ranging from $2n=24$ to $2n=72$ in Mexico and South America. Welcome, too, is the inclusion of two maps of South America showing the distribution of the various cultivated types; one of the diploid and pentaploid groups, the other of the triploid and tetraploid—a reminder that there is no simple answer to the question which, in days not so long ago, was so frequently put: What is, or was, the original wild species from which our cultivated potato was derived?

Hawkes, who is in full agreement with the reviewer as to the derivation of the European potato from an *S. andigenum* type, a native of Colombia or thereabouts, throws a welcome light on the origin of *S. andigenum* and the so-called *S. tuberosum* s.str. of Chile. He is of opinion that *S. andigenum* arose from the doubling of a cultivated diploid species, probably *S. stenotomum*, and that this transformation occurred in the Lake Titicaca region of Bolivia-Peru during the early days of man's penetration there. He is also inclined to think that the Chilean *S. tuberosum* s.str. derives essentially from the same source, but that it has undergone certain adaptive changes as the result of isolation and of selection in favour of long-day types, attuned to the humid climatic conditions there obtaining.

In his Introduction the author explains why the work has not been expanded into a monograph, and in that decision, in these days of stress, we must reluctantly acquiesce. It is to be hoped that before very long Dr. Hawkes, so well qualified for this work, will complete the task of describing and evaluating the fascinating treasures he has helped to win from the mighty Andes, the initial steps of which he has so well and truly established. It is to be hoped, too, that nothing will be allowed to prevent a presentation of the taxonomic, ecologic, genetic and physiological tale of the tuber-bearing Solanums, later, in book form.

REDCLIFFE N. SALAMAN.

PHOTOPERIODISM IN THE POTATO. By C. M. DRIVER and J. G. HAWKES. (Cambridge, Imperial Bureau of Plant Breeding and Genetics, 1943, pp. 36, 2s. 6d. Obtainable, post free, from Imp. Ag. Bur., Agricultural Research Buildings, Aberystwyth.)

In addition to being the source of energy utilized by the green plant for manufacturing its food, light exerts an influence on its general growth and development. Under natural conditions plants are exposed to alternating periods of light and darkness (day and night) and these periods vary in length according to season and latitude. This alternation is referred to as photoperiodism and the present bulletin is concerned with the effect of length or shortness of day on the yield and maturity of over seventy varieties of cultivated potatoes, derived from seven species, collected from the department of Puno in the Lake Titicaca region of the Peru-Bolivian Andes. A single Mexican species (*Solanum demissum*) is also included.

In Part I (General), the first of the two authors provides a detailed survey of the literature published during the past twenty years or so on the subject, the sources of the papers consulted

being listed at the end of the bulletin. This survey embraces the experimental methods employed and the effect of temperature on photoperiodism. It covers the effect of the photoperiod on vegetative growth, flowering and formation of stolons and tubers as well as on maturity. Photoperiodism in relation to plant physiology is discussed and work on its inheritance is reviewed. Finally, previous investigations on the response of certain South American species of potatoes to photoperiodism are summarized.

In Part II (The Photoperiodic Reactions of some South American Potatoes) the second author gives a full account of his experimental work, carried out at Cambridge in the years 1938, 1939 and 1940. Except in regard to the periods of exposure to light the plants were grown under conditions as similar as possible. The tubers were planted in March and grown on in 7 in. pots in a greenhouse. For shortening the "day" the plants were wheeled into a dark shed, contiguous to the glasshouse. These plants were exposed to daylight from 8.30 a.m. to 5 p.m., a period of $8\frac{1}{2}$ hours only, which is considerably shorter than that which they would experience in their native locality (11-13 hours). The long-day plants were exposed from sunrise to sunset, about 13 hours in April and 16 hours in June.

The results obtained, which are fully discussed, are summarized in three Tables. In the first, the tuber weights are recorded for the three years and the tuber number for 1940. In addition, the "photoperiodic index" for tuber weight and number is given for each variety. This index is obtained by dividing the short-day yields by the long-day ones and multiplying by 100. From it the plants can be classified into long- or short-day varieties, in which the yields differ quite considerably, or included in a day-neutral class, in which the yields do not differ markedly. In the second and third Tables the results in regard to stolon production, made under long-day conditions, maturity, height of plants and flowering, under long- and short-day conditions, are presented.

Most of the species studied (including *S. demissum*) were definitely short-day ones, some of them giving no tubers at all under long-day conditions. Short-days increased tuber weight rather than number. Many long-day stolons were lengthy with tips protruding above the soil; short-day stolons were few, short and subterranean. The varieties tested required on the average $1\frac{1}{2}$ times as long to mature under long- as under short-day conditions. Short-days depressed flowering, but this was perhaps due to a reduction in total quantity of light received rather than to photoperiodism. It is suggested that photoperiodism influences mainly a plant's dominant mode of reproduction; in the potato, therefore, tuber rather than flower production.

The bulletin constitutes a valuable addition to our knowledge of the response of potato varieties to length of day, and it will be of special interest to those engaged in breeding new varieties adapted to particular conditions of climate or geographical range.

G.H.P.

A DICTIONARY OF THE FUNGI. By G. C. AINSWORTH and G. R. BISBY. (Kew, The Imperial Mycological Institute, 1943, pp. 359 + viii, 138 figs., 20s. or U.S. $4.60.)

To appreciate the relevance of the present work to pomologists in general and, in particular, to those concerned with the diseases of fruit trees and other horticultural crops, it has only to be recollected that of the many parasitic diseases at present identified the great majority are of fungus origin. Its appearance may be taken as both a sign and a symbol of an increasing awareness of the vital relationship between a sound fungal taxonomy, on the one hand, and plant pathology and the branches of applied mycology, on the other; and it is significant that one of the authors has worked considerably in the field of plant pathology and the other in that of pure taxonomy. The result is a work which, following its acknowledged prototype, Willis's *Dictionary of Flowering Plants and Ferns*, adequately covers with all the convenience of rapid reference which its format implies, the whole range and scope of mycological science, pure and applied.

The backbone of the Dictionary is " a list of all the generic names of Fungi (Eumycetes and Myxothallophyta but not Bacteria and Lichens) which have been in use to the end of 1939 ", together with the number and distribution of their species. Short accounts are also given of the larger divisions (Families, Orders, etc.) of the Fungi and also of Bacteria and Lichens ; and G. W. Martin's illustrated " Key to the Families of Fungi " is included as an Appendix.

The present need for a critical revision of fungus genera and species is apparent from the fact that " About half the 7,000 generic names are listed as synonyms ". This thorny subject is efficiently and impartially covered by the authors so that, as Dr. S. P. Wiltshire comments in his Introduction to the work, " their conclusions reasonably reflect current mycological opinion ".

This strong systematic backbone is clothed with a wealth of references and with short articles on all conceivable subjects relevant to Mycology, from general and often recondite and picturesque topics, such as "Smell", to mycological Plant Pathology and the applied branches such as Medical and Industrial Mycology. References to fungus diseases of fruit and other crops and plants occur both under the names of fungus genera, where important parasitic species are named together with the common name of the disease, and separately, under the common names of the diseases which (presumably for the sake of economy of space) are usually quoted generically, as for example : "*Shot-Hole*, a leaf spot disease characterized by holes made by the dead parts dropping out ;—of *Antirrhinum* (*Heteropatella antirrhini* Buddin & Wakef.) ; of peach (*Clasterosporium carpophilum* (Lév.) Aderh.) ; of plum (*Pseudomonas prunicola* Wormald)." This quotation illustrates both how the work enables, in small compass, a rapid review of analagous types of disease with their often very differing causal fungi and bacteria to be made, and also reveals the small errors and omissions inevitable in the first edition of a work of this encyclopaedic nature. The bacterium *Pseudomonas mors-prunorum* Wormald is the more important cause of Shot-Hole in plums. The reference to important fruit and other plant diseases are also sometimes incomplete. Thus of the species of Coniothyrium it is said, " Some are minor plant pathogens ". But at least one species, *Leptosphaeria Coniothyrium* (Fuckel) Sacc. (*C. Fuckelii*) causes a serious disease of rose and raspberry. A cross reference to *Leptosphaeria* would have helped here.

It is not yet generally or sufficiently recognized that a determination of the pathogen's true taxonomic relationship is absolutely essential in research on any parasitic disease. Thus the availability of invaluable data on the etiology and control of some diseases has long been restricted because the synonomy of the pathogenic fungi has not been adequately investigated, e.g. the same fungus has been studied in another country, *but under another name*. The present work should go far towards the elimination of such dissipations of research energy and it provides a means towards a closer union of sound fungus taxonomy and the practice of mycological plant pathology.

R.V.H.

LIST OF COMMON BRITISH PLANT DISEASES. Compiled by the Plant Pathology Committee of the British Mycological Society. (Cambridge University Press, 1944, pp. 61, 5s. net.)

The new *List of Common British Plant Diseases* is the third edition of the original *List of Common Names of British Plant Diseases*, compiled in 1928 by the Plant Pathology Sub-Committee of the British Mycological Society, and published in the following year. The primary object of the list was to encourage the use, throughout the British Isles and the Empire, of a single common name for each disease, so as to avoid confusion in naming the diseases, particularly in popular articles. The new edition has been remodelled, revised, and enlarged. The host plants, instead of being arranged in groups (cereals, vegetables, etc.) as in previous editions,

are listed alphabetically, according to the names by which they are best known; and tree diseases are now included.

The slight change in the title has been adopted to put more emphasis on the scientific names of the parasites. These names, quoted in accordance with the International Rules of Botanical Nomenclature, have been found to be quite as useful to plant pathologists as the common names of the diseases; and now over forty Offices, Societies and Institutes in the British Isles have agreed to use in their official publications the scientific names of fungi given in the list. The editors of *The Journal of Pomology* have subscribed to this agreement and contributors to this Journal should make a note of this. The discussion, in the Introduction, on the changes made from time to time in the scientific names, will be instructive to plant pathologists and others who are interested in the nomenclature of the pathogens.

The diseases mentioned are those caused by fungi, bacteria, and viruses, and others of a non-parasitic nature. As many of these diseases are prevalent abroad, the names used for them in foreign countries are given. An innovation in the present edition is the inclusion of the Russian names for many of the diseases; this hints at a closer collaboration with our eastern ally in the study of those diseases of crop plants common to the two countries, a collaboration which will be all the more urgent since agricultural and horticultural research in all its branches is likely to be checked for some years in the rest of Europe.

In this connection it may be pointed out that the list does not include diseases of Sunflowers; this plant is cultivated in Russia on a fairly large scale for the oil in its seeds, and an attempt is being made to explore the possibility of cultivating it for that purpose in England (see *Journ. Minist. Agric.*, Vol. L, No. 11, Feb., 1944, 517-521). Mould (*Botrytis cinerea*) and Sclerotinia (? *sclerotiorum*) were noted on the trial plots; the latter is known to cause considerable loss to the crop in Russia.

The book concludes with a " List of Authors' Names and Abbreviations ", an " Index of Foreign Common Names " (with a separate list of nearly 100 Russian names), and an " Index of Latin Names ".

The Secretary of the Plant Pathological Committee invites suggestions from plant pathologists and mycologists, for the improvement of the List, for use when a further edition is called for.

H.W.

THE CHEMICAL TESTING OF PLANT NUTRIENT SOLUTIONS. By G. S. FAWCETT and R. H. STOUGHTON. (The Tintometer Ltd., Salisbury, Wilts., 1944, pp. 86, 8s. 6d.)

The practical possibilities of nutrient solution methods of growing plants without soil depend, among other things, on having simple rapid methods of analysis which the grower can use for periodically testing the composition of his nutrient solutions. The present book provides the grower with information on laboratory procedure, apparatus required and methods of analysis which have been found useful for the purpose. Simplicity of operation and rapidity of analysis are requirements which impose limitations on the choice of methods and on the accuracy of the results. The authors are quite well aware of this and it is a pleasing feature of the book that difficulties and the merits and defects of each method are well presented.

Separation of nutrients before their estimation is not attempted. Colorimetric or turbidimetric methods of analysis are applied directly to the nutrient solution, and compensation for interfering substances is made by calibration with standard solutions containing all the other ingredients of the nutrient solution and by adding reagents which have the effect of suppressing the interfering substance. Colorimetric tests are used for the estimation of pH, nitrate nitrogen, phosphate, magnesium, iron, manganese, and boron, and turbidimetric tests are used for potassium and calcium. Rough methods for copper and zinc and a tentative colorimetric method for potassium are given in appendices at the end of the book.

In the colorimetric methods use is made of the All Purposes Lovibond Comparator and of discs containing a range of glass colour standards. Procedure is built around the use of this apparatus. Glass colour standards save time, labour and apparatus, but they do not make tests on standard solutions unnecessary, as one might suppose from the book, for the latter are needed to assess the effectiveness of the reagents used.

In the turbidimetric methods and the test for boron, comparison with liquid standards is made in black-sided micro-test-tubes, and simple apparatus is described for this purpose. Line-charts, so widely used in rapid turbidimetric tests, are rejected as fundamentally unsound and the proposed method should be an improvement on them.

The reagents used in the tests have been known for many years but only comparatively recently has their use been extended from detection of the element to its quantitative estimation. A considerable amount of research has been necessary in developing quantitative methods and it would improve the quality of the book if more numerous references were included, preferably as footnotes to the text pages. Notes on the action of some of the reagents added to deal with interfering substances, would also be helpful and would save a lot of searching of the literature on the part of those using the tests for the first time. The authors may have considered it inadvisable or unnecessary to include such information in a book written primarily for the practical grower, but the book will undoubtedly interest others with more technical knowledge, and it is from these that suggestions for improving the methods will eventually come.

While it is not the function of the book to deal with interpretation of the tests it might be helpful to include in an appendix an example of the calculation of the quantity of nutrient salt to be added and the procedure to be followed in order to correct a deficiency in, say, 100 gallons of nutrient solution.

The book is well planned and the typography is excellent ; only one error in spelling was noted, " phosphorous " for " phosphorus " on p. 9. The binding and paper are good considering wartime conditions.

N.H.P.

PROPAGATION BY CUTTINGS AND LAYERS. Recent work and its application with special reference to pome and stone fruits. By R. J. GARNER. (Imp. Bur. of Hort. and Plantation Crops, Technical Communication No. 14, 1944. East Malling Research Station. Price 3s. 6d., obtainable from Imperial Agricultural Bureaux, Central Sales Branch, Penglais, Aberystwyth.)

This bulletin maintains the high standard set by previous communications in this series. The whole question of propagation is dealt with in a systematic and comprehensive way ; and the vast amount of information enclosed in this slender volume is presented with such skill that the reader, though approaching the subject in entire ignorance, will in the course of reading, gain a thorough insight into the problems of propagation.

The book falls into two parts, the first dealing with the more theoretical aspects, the second with a lucid account of the methods in use at the East Malling Research Station, and largely developed there by the author and his predecessors. The first part is introduced by a succinct historical sketch and states clearly the author's plan of presentation. By adhering to this plan the review of literature is reduced to an orderly array under three main headings.

The first section deals with the source of material for cuttings, including the age and condition of the parent plant, desirable and undesirable characteristics in the parent, the type of material to be selected, and the time at which cuttings should be taken. The second section concerns the handling of cuttings prior to planting, and it details methods of control by treatment with synthetic growth substances and other materials, the modification of internal conditions of the cutting by adjusting the external factors of light, temperature, and humidity, and various cultural expedients such as wounding and nurse grafting. The third section deals with planting

and subsequent treatment of cuttings, and discusses the effects of types of rooting media, spacing, methods of planting and the level of external factors together with the use of fertilizers, growth hormones and other chemical agencies. The propagation of layers is next presented under the heads of stooling, layering, and marcotting, and the section ends with a brief account of other methods of propagation. Each separate section is provided with a summary embodying the salient information found in the literature.

The practical details of operations occupy the second part of the bulletin, and will appeal particularly to the practical horticulturist, and make available to everyone the results of years of research by the author and his associates at East Malling.

In the application of scientific methods to practical affairs there are always two ways of procedure open, namely the empirical approach and the method of advance by hypothesis and crucial experiment. In a field such as vegetative propagation, which, as an art, has apparently been practised from the dawn of human history, the method of trial and error will have established empirical methods far in advance of theory. Indeed, plant physiology is even now scarcely in a position to formulate, let alone elucidate, the problems underlying the practice. But striking advances have been and are being made in the field of experimental morphology, to which the science of propagation properly belongs; and the recent discoveries of photoperiodism, vernalization, and growth hormones lend an added impetus to research and a livelier interest to the phenomena of regeneration. The academic researcher is often handicapped by ignorance of the facts, while the practical horticulturist is often unconcerned with the deeper issues. This bulletin supplies a real need in bringing to the knowledge of each the achievements of the other. Mr. Garner has shown throughout a lively interest in theory, and a mastery of practice, and nowhere more clearly than in the "Thoughts on future research" with which the bulletin closes. To the reviewer, the dearth of general theory, adequate to direct the analytical investigations called for, is all too clear. The carbohydrate/nitrogen ratio hypothesis still remains almost the sole guide in unravelling the complexity of the phenomena of propagation. Mr. Garner's programme of research is comprehensive but still in the main empirical. The clue, in the reviewer's opinion, lies in long range research directed to two ends: first, the problems centring around meristematic organization and activity, and second, the functional organization, whereby the primary physiological processes are co-ordinated. This may appear too vague an aim and as this is not the occasion to lend it definiteness by translation into physiological and biochemical terms, it must necessarily remain so. The task is formidable but until achieved, propagation will remain an art, and research do little more than confirm previous practice and suggest new empirical methods. The appraisement of Mr. Garner's work in the foreword by Dr. R. G. Hatton, F.R.S., is so just that nothing more in this kind need be added, other than to acknowledge the profit and pleasure the reviewer has derived from this excellent work.

F. G. Gregory.

SOIL AND PLANT ANALYSIS. A laboratory manual of methods for the examination of soils and the determination of the inorganic constituents of plants. By C. S. Piper. (University of Adelaide, 1942, pp. 368, 19 figs., price 15s. Aust.)

Part I of this book (250 pages) is devoted to methods for the examination of soils, and Part II (112 pages) to methods for the determination of the inorganic constituents of plants. Part I follows on an earlier publication entitled *Methods for the examination of soils* (C.S.I.R. Pamphlet No. 8, by Professor J. A. Prescott and the author of the present book) and incorporates additions and improvements resulting from discussions with chemists working throughout Australia and New Zealand and in other parts of the world. It is stated in the preface: "With the exception of two or three of the less common determinations, all of the methods included . . . are in actual use in the laboratories." In the introduction the writer states that in the choice of methods to be employed, "accuracy and reproducibility of the values obtained should

be of the first importance and the results should be directly comparable with results obtained in other laboratories ". The following two statements suggest that the writer looks on plant analysis as subsidiary to soil analysis : " In many fertility problems the information gained from a study of the soil can be considerably enhanced by the analysis of the plants growing on the soil." " The analysis of a range of plant species growing on a given soil type can prove a useful guide to the relative availability of the different mineral elements in that soil. Plant analysis has been found particularly valuable in regard to the availability of the trace elements such as manganese, copper, zinc and boron."

The Neubauer, Mitcherlich and Aspergillus methods are mentioned but are not described.

As its title suggests, the book is confined to soil and plant analysis, and interpretation of results is not considered. It is clearly written and is full of valuable practical details. It gives, moreover, most of the latest methods, including those for the trace elements : boron, cobalt, copper, iron, manganese, molybdenum and zinc.

W.A.R.

THE CITRUS INDUSTRY. Vol. I. History, botany and breeding. Edited by H. J. WEBBER and L. D. BATCHELOR. (University of California Press, 1943, pp. 1028, $7.50.)

This volume, the first of a projected trilogy on the Citrus industry of the world, provides an extended summary of the most important information available on Citrus history and botany. It includes in its pages discussions on climatology, geography, species, varieties, anatomy, physiology, nutrition, embryology, genetics and breeding, covering, in fact, the life history of Citrus plants, with special relation to their commercial production. If the spotlight seems to be concentrated chiefly on California it is not unnatural, for the authors live there, most of the research is done there, while the stream-lined efficiency of Californian production and marketing methods forms an ever-present stimulus to competitors in more leisured lands. Volumes II and III, yet to come, will deal respectively with the production of the crop, and with harvesting, marketing and utilization. They will bear titles to this effect. The present treatise is in reality a symposium, a number of workers of renown in Citrus research contributing chapters bearing on the subjects of which they have made a particular study. Such well-known names as Swingle, Shamel, Bartholemew and Reed, Frost and Chapman, and Kelley are sufficient guarantee that the ground has been well and truly covered. The senior editor, H. J. Webber, is responsible for four of the ten chapters, and his observations on the history of the industry, climatology, geography and related matters are at once erudite and very readable. Of considerable importance is the monograph of Citrus species by W. T. Swingle entitled " The botany of Citrus and its wild relatives of the orange sub-family ", because there, for the first time for many years, is presented a connected account of the whole orange sub-family, supplemented by remarks on the geographical distribution and probable evolutionary history of the 33 genera treated, and on the possible economic characters of such species as possess them. Cultivated varieties of Citrus are described by Webber and are here confined to some 215 of the most important varieties grown in the United States. Notes on origin are given. Though all modern commercial varieties are of fairly recent date and though descended from the old types described by early writers, they doubtless differ from them genetically. Bartholemew and Reed give a sufficiently full account of Citrus morphology, histology and physiology. The chapter on nutrition, contributed by Chapman and Kelley, is concerned with the mineral composition of Citrus and with its plant-food requirements, special emphasis being laid on the effects of deficiencies and excesses, with a brief note on soil reaction. More intimate physiological reactions such as the influence of one ion of absorption on another, translocation and mobility of nutrients within the plant and kindred topics, of which positive knowledge is scant, are not discussed. H. B. Frost deals with seed reproduction, chiefly by way of a description of the reproductive parts and processes ; and in a second chapter he summarizes and interprets the available evidence on

heredity, applying what knowledge there is to the problems of the production of improved horticultural varieties. The concluding chapter, " Bud variation and bud selection ", is very properly by A. D. Shamel, than whom there is no greater authority. With a number of authors contributing, some inconsistencies may be expected and duly appear, but they can be fully excused, it is explained, by reason of the data available being open to different interpretations. The production of the book in terms of paper, illustrations, type and binding is fully up to the best pre-war standards, though presumably there has been some delay in publication, the preface being dated 1938, while the publishing date is five years later. The list of nearly 1,200 references has, however, been brought up to date, at least as regards recent papers by the contributors.

G.St.C.F.

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2. Authors of papers containing extensive **numerical observations** should consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from Burlington House, London, W.1. Price 6d. Arrangements have been made whereby original data too extensive for complete publication may be deposited at the British Museum (Natural History), London, S.W.7, or with the Royal Society of Edinburgh, 22 George Street, and thus be available for consultation by other workers. Full bibliographical reference to the publication in which the results are summarized must be supplied with the data.

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